

# Minifors 2

## Operating Manual



CE

We bring life to your laboratory. **INFORS HT**

# Minifors 2 – Unbeatable in its class.

**Minifors 2** – Rev. 2.1  
Bench-Top Bioreactor  
SW: 3.2 / FW: 2.04

Doc-Nr. 78972  
EN V.04.03 - Original

---

This operating manual can also be  
found online at:  
[www.infors-ht.com/en/minifors2](http://www.infors-ht.com/en/minifors2)



**Infors AG**  
Headoffice, Switzerland  
Rittergasse 27  
CH-4103 Bottmingen

T +41 (0)61 425 77 00

[info@infors-ht.com](mailto:info@infors-ht.com)  
[service@infors-ht.com](mailto:service@infors-ht.com)

**Table of Contents**

**1 General Information..... 9**

1.1 About this Manual ..... 9

1.2 Explanation of Special Notices ..... 10

    1.2.1 Warning Notices ..... 10

    1.2.2 Other Notices..... 10

1.3 Device Identification (Standard Identification Plate) ..... 11

1.4 Declaration of Conformity ..... 11

1.5 Customer Service and Services ..... 11

**2 Safety and Responsibility ..... 12**

2.1 Intended Use, Incorrect Use and Misuse ..... 12

2.2 Qualified Personnel ..... 13

    2.2.1 Provider ..... 13

    2.2.2 User ..... 13

    2.2.3 Operator ..... 14

2.3 Unauthorised Persons..... 15

2.4 Responsibility of the Provider..... 15

2.5 General Hazards..... 16

    2.5.1 Electrical Current ..... 16

    2.5.2 Unauthorised Spare Parts and Accessories ..... 16

2.6 Particular Hazards..... 16

    2.6.1 Hot Surfaces ..... 17

    2.6.2 Dangerous Gases ..... 17

    2.6.3 Flammable or Explosive Substances..... 17

    2.6.4 Corrosive or Toxic Substances ..... 18

    2.6.5 Bioactive Substances or Pathogenic Organisms  
..... 18

    2.6.6 Overpressure or Vacuum..... 18

2.7 Warning Symbols on the Device ..... 18

2.8 Declaration of Decontamination..... 19

**3 Setup and Function ..... 20**

3.1 Basic Unit ..... 20

    3.1.1 Power Switch..... 21

    3.1.2 LED Strip – Status Indicator ..... 21

    3.1.3 Pumps ..... 22

    3.1.4 Identification Plate ..... 23

    3.1.5 Mains Connection and Device Fuses ..... 23

    3.1.6 Water Connections ..... 24

    3.1.7 Gas Connections ..... 24

    3.1.8 Signal Connections ..... 25

## Table of Contents

3.1.9	Motor Cable Connection .....	26
3.1.10	Connections for Sensors (Sensor Cables).....	26
3.1.11	Gassing Connections (Sparger & Head Space)	27
3.1.12	Connections and Water Flow Control Valve for the Exit Gas Cooler .....	29
3.2	Operating Panel .....	30
3.3	Culture Vessel .....	31
3.3.1	Top Plate .....	32
3.3.2	Ports in the Vessel Top Plate and their Configuration .....	33
3.3.3	Vessel Top Plate, DN 90 .....	33
3.3.4	Vessel Top Plate, DN 115 .....	34
3.3.5	Vessel Top Plate, DN 145 .....	35
3.4	Temperature Control System .....	36
3.5	Stirrer .....	37
3.5.1	Motor .....	37
3.5.2	Impellers .....	38
3.6	Gassing System .....	39
3.6.1	Gas Entry .....	40
3.6.2	Exit Gas .....	40
3.7	pH Control .....	41
3.7.1	Measurement System .....	41
3.8	pO <sub>2</sub> Control.....	42
3.8.1	Measurement System .....	42
3.9	Antifoam Control.....	44
3.9.1	Antifoam Sensor .....	44
<b>4</b>	<b>Options.....</b>	<b>45</b>
4.1	Turbidity Measurement .....	45
4.1.1	Calibrating the Sensor .....	46
4.1.2	Mounting the Sensor .....	47
4.1.3	Interferences Turbidity Measurement.....	48
4.2	Exit Gas Analysis.....	48
4.2.1	Gas Sensor.....	48
4.2.2	Connecting the Gas Sensor.....	49
4.2.3	Calibrating the Gas Sensor.....	50
4.2.4	Replacing the BlueVary Gas Sensor Cartridge.	50
<b>5</b>	<b>Accessories .....</b>	<b>51</b>
5.1	Cone Plug for Drive Hub .....	52
5.2	Sparger .....	52
5.3	Baffles.....	53

**Table of Contents**

5.4 Blanking Plugs..... 53

5.5 Clamping Adapters and Fastening Screws ..... 54

5.6 Electrode Holders ..... 55

5.7 Addition Port Adapters and Feed Needles ..... 55

5.8 Septum Collar ..... 57

5.9 Dip Tubes..... 57

5.10 Immersion Pocket for Temperature Sensor (Pt100) ..... 58

5.11 Exit Gas Cooler..... 58

5.12 Cold Finger ..... 59

5.13 Reagent Bottles ..... 61

5.14 Sampling System Super Safe Sampler ..... 61

5.15 Pump Heads..... 65

5.16 Vessel Holder with Built-in Holder for Reagent Bottles and Pumps..... 65

5.17 Sterile Filters..... 66

5.18 Hoses and Accessories..... 68

5.19 O-Rings and Gaskets ..... 69

5.20 Inoculation Accessories and Tools..... 69

5.21 Starter Kit..... 70

5.22 Service Sets ..... 70

5.23 Auxiliary Supplies ..... 70

**6 Transport and Storage ..... 71**

6.1 Transport ..... 71

6.2 Storage ..... 72

**7 Installation and Initial Operation..... 73**

7.1 General Location Requirements for Installation..... 73

7.2 Minimum Distances..... 73

7.3 Connecting the Device to On-Site Supply Lines ..... 73

7.3.1 Power Supply..... 74

7.3.2 Water Supply and Return..... 74

7.3.3 Gas Supply..... 75

7.3.4 Exit Gas ..... 76

7.4 Connecting the Motor Cable..... 76

7.5 Test Run..... 77

7.5.1 Preparation Test Run..... 78

7.5.2 Cooling System..... 79

7.5.3 Stirring..... 79

7.5.4 Heating and Adjusting Temperature ..... 80

7.5.5 Gassing..... 80

7.5.6 End of Test ..... 81

## Table of Contents

<b>8</b>	<b>Before Cultivation .....</b>	<b>82</b>
8.1	Preparing and Autoclaving the Culture Vessel .....	82
8.1.1	Checking Gaskets (O-Rings) .....	82
8.1.2	Mounting the Impellers.....	83
8.1.3	Mounting Dip Tubes and Spargers.....	85
8.1.4	Inserting the Vessel into the Vessel Holder .....	86
8.1.5	Inserting the Baffles .....	87
8.1.6	Moistening/Filling the Culture Vessel.....	87
8.1.7	Fitting the Vessel Top Plate .....	88
8.1.8	Mounting the Blanking Plugs.....	89
8.1.9	Mounting Addition Port Adapters.....	90
8.1.10	Mounting the Feed Needle(s).....	90
8.1.11	Mounting the Immersion Pocket for Temperature Sensor (Pt100).....	91
8.1.12	Equipping the Port with a Septum Collar and Septum for Inoculation .....	92
8.1.13	Preparing the Dip Tube/Addition Port Adapter for Inoculation .....	93
8.1.14	Mounting the Exit Gas Cooler.....	93
8.1.15	Mounting the Cold Finger.....	94
8.1.16	Preparing the Sensors .....	94
	8.1.16.1 Calibrating the pH Sensor.....	95
	8.1.16.2 Mounting a Sensor into a 12 mm Port.....	95
	8.1.16.3 Mounting Sensors with Electrode Holder .....	96
	8.1.16.4 Mounting the Antifoam Sensor .....	98
8.1.17	Preparing the Super Safe Sampler .....	100
8.1.18	Mounting the Sparger Hose and the Inlet Air Filter .....	101
8.1.19	Mounting the Hose and Inlet Air Filter for Head Space Gassing.....	102
8.1.20	Preparing the Reagent Bottles, Pumps and Hoses.....	103
8.1.21	Sterile Hose Connections .....	106
8.1.22	Setting the Pumps .....	106
8.1.23	Removing the Pump Heads.....	107
8.1.24	Fitting the Cone Plug for Drive Hub .....	107
8.1.25	Checklist Before Autoclaving .....	108
8.1.26	Autoclaving .....	109
8.2	Connecting the Culture Vessel and Preparing the Cultivation .....	111

**Table of Contents**

8.2.1	Hang the Culture Vessel in Place and Fit the Pump Heads .....	111
8.2.2	Filling the Reagent Hoses .....	112
8.2.3	Connecting the Gassing.....	112
8.2.4	Connecting the Exit Gas Cooler .....	113
8.2.5	Connecting the Cold Finger .....	114
8.2.6	Coupling the Motor .....	114
8.2.7	Filling the Culture Vessel.....	115
8.2.8	Connecting the Temperature Sensor (Pt100).....	115
8.2.9	Connecting the Antifoam Sensor .....	116
8.2.10	Connecting the pH Sensor .....	117
8.2.11	Connecting the pO <sub>2</sub> Sensor.....	117
8.2.12	Calibrating the pO <sub>2</sub> Sensor.....	118
8.2.13	Checking the Hoses and Hose Connections .	118
<b>9</b>	<b>Cultivation.....</b>	<b>119</b>
9.1	Preparing the Medium .....	119
9.2	Sampling.....	120
9.3	Inoculation .....	124
9.3.1	Inoculation with a Syringe.....	125
9.3.2	Inoculation Using Dip Tube / Addition Port Adapter .....	125
9.4	Harvest.....	125
9.5	Emptying the Culture Vessel.....	126
9.6	Emptying the Reagent Hoses.....	127
9.7	Switching off the Device .....	127
9.8	Autoclaving the Culture Vessel After Cultivation .....	128
<b>10</b>	<b>Operation .....</b>	<b>130</b>
10.1	Screen Areas, Menu Navigation and Control Elements	130
10.1.1	Main Screen.....	132
10.1.2	EDIT VIEW.....	134
10.1.3	START BATCH / INOCULATE / STOP BATCH .	134
10.1.4	SAMPLE NOW.....	136
10.2	Menus for System Settings .....	137
10.2.1	VESSEL TYPE – Selecting a Culture Vessel ....	138
10.2.2	APPEARANCE – Display Settings .....	139
10.2.3	NETWORK SETTINGS .....	141
10.2.4	E COMMUNICATION – Communication Settings .....	142
10.2.5	USB Data Export and Import from a USB Stick .....	143
10.2.6	SYSTEM INFO – System Information.....	146

## Table of Contents

10.3	Parameter - Parameter Groups .....	148
10.3.1	Parameters – Displays and Functions.....	149
10.3.2	SETPOINT - Setting the Setpoint.....	150
10.3.3	Parameter Alarms .....	152
10.3.4	Cascades .....	154
10.4	MAIN Parameter Group.....	155
10.4.1	Temperature .....	155
10.4.2	Stirrer .....	155
10.4.3	pH .....	155
10.4.4	pO <sub>2</sub> .....	157
10.4.5	Total Flow.....	160
10.4.6	GasMix .....	161
10.4.7	Foam .....	162
10.5	EXTENDED Parameter Group.....	164
10.5.1	Balance (Optional) .....	164
10.5.2	Air Flow, Gas2 Flow, O <sub>2</sub> Flow, N <sub>2</sub> Flow, Air Headspace, CO <sub>2</sub> Flow .....	165
10.5.3	Turbidity (Optional).....	166
10.5.4	Analog IO1 & Analog IO2 .....	166
10.6	EXIT GAS Parameter Group.....	168
10.6.1	Exit Gas O <sub>2</sub> .....	168
10.6.2	Exit Gas CO <sub>2</sub> .....	168
10.7	PUMPS Parameter Group - General Information .....	169
10.7.1	Configuring the Pumps.....	170
10.7.2	Pump1 - Acid or Additional Feed Solution....	172
10.7.3	Pump2 - Base or Additional Feed Solution ...	172
10.7.4	Pump3 - Antifoam, Level or Additional Feed Solution .....	173
10.7.5	Pump4 - Feed Solution.....	174
10.7.6	AUTO FILL/EMPTY – Automatically Filling/Emptying Pump Hoses .....	176
10.8	Calibration .....	177
10.8.1	Calibrating the pH Sensor - General Information .....	177
10.8.2	Calibrating the pH Sensor - Procedure .....	178
10.8.3	pH Sensor Product Calibration .....	182
10.8.4	Calibrating the pO <sub>2</sub> Sensor - General Information.....	185
10.8.5	Calibrating the pO <sub>2</sub> sensor - Procedure.....	185
10.8.6	Calibrating the Turbidity Sensor - General Information.....	189
10.8.7	Calibrating the Turbidity Sensor - Procedure	189

**Table of Contents**

10.9 PID Controller – Basic Principle ..... 191

    10.9.1 Table with Setting Values for PID Controller. 191

    10.9.2 Useful Information for Changing PID Controller Settings ..... 191

    10.9.3 Adjusting PID Settings..... 192

10.10 Alarms – Equipment Alarm Menu..... 192

**11 Cleaning and Maintenance..... 194**

11.1 Cleaning Agent and Disinfectant..... 194

11.2 Cleaning the Culture Vessel - Routine Cleaning..... 195

11.3 Removing the Vessel Top Plate and Accessories..... 196

    11.3.1 Removing the Exit Gas Cooler..... 196

    11.3.2 Removing the Sensors..... 197

    11.3.3 Removing Hoses, Filters and Pump Heads .... 197

    11.3.4 Removing Blanking Plugs ..... 198

    11.3.5 Removing the Septum Collar and Septum.... 198

    11.3.6 Removing Addition Port Adapters, Feed Needle and Temperature Sensor Immersion Pocket . 199

    11.3.7 Removing the Vessel Top Plate..... 199

    11.3.8 Removing the Sparger and the Dip Tube(s) ..200

    11.3.9 Removing the Impeller(s)..... 200

11.4 Cleaning and Storing Individual Parts ..... 201

11.5 Cleaning the Sensors..... 202

11.6 Cleaning the Hoses and Pump Heads ..... 202

11.7 Cleaning the Super Safe Sampler ..... 202

11.8 Cleaning the Exit Gas Cooler ..... 203

11.9 Cleaning the Basic Unit and Operating Panel..... 203

11.10 Maintenance Plan..... 204

11.11 Decalcifying the Device ..... 205

**12 Interferences ..... 207**

12.1 Interferences Basic Unit and Operating Panel ..... 207

12.2 Interferences Drive System ..... 208

12.3 Interferences Temperature Control System ..... 209

12.4 Interferences Gassing System ..... 210

12.5 Interferences pH-System..... 211

12.6 Interferences pO<sub>2</sub> System..... 213

12.7 Interferences Antifoam/Level Sensor and Antifoam Pumps ..... 214

12.8 Interferences Addition of Nutrient Solution (Feed Pump) ..... 215

12.9 Replacing Device Fuses..... 215

12.10 Behaviour in Case of Power Interruption ..... 216

## Table of Contents

12.11	Returning for Repair .....	216
<b>13</b>	<b>Disassembly and Disposal.....</b>	<b>217</b>
13.1	Disassembly.....	217
13.2	Disposal .....	218
<b>14</b>	<b>Technical Data.....</b>	<b>219</b>
14.1	Dimensions .....	219
14.1.1	Front View Device .....	219
14.1.2	Top View Device .....	220
14.1.3	Culture Vessel.....	221
14.2	Weights (netto) .....	222
14.3	Connection Requirements .....	222
14.3.1	Electrical .....	222
14.3.2	Water .....	222
14.3.3	Gas.....	223
14.4	Specifications .....	223
14.4.1	Operating Panel.....	223
14.4.2	Culture Vessels .....	223
14.4.3	Stirrer .....	225
14.4.4	Temperature.....	227
14.4.5	Gassing.....	228
14.4.6	pH .....	229
14.4.7	pO <sub>2</sub> .....	230
14.4.8	Antifoam .....	230
14.4.9	Pumps .....	231
14.5	Operating Conditions .....	232
14.6	Emissions .....	232
14.7	Auxiliary Supplies .....	232
<b>15</b>	<b>EC-Declaration of Conformity .....</b>	<b>233</b>

# 1 General Information

## 1.1 About this Manual

This manual enables the safe and efficient handling of the device.

All the information and instructions in this operating manual comply with the current standards, legal regulations, the latest technological and scientific developments and the knowledge gained from the manufacturer's many years of experience in this field.



**This operating manual is a component part of the device. It must be kept near to the device and must be accessible to the operators at all times.**

The users must read the operating manual thoroughly and fully understand its contents before beginning any work.

Adhering to all the safety and operating instructions in this manual is essential to ensure that work is carried out safely.

The scope of delivery may differ from the explanations, descriptions and figures in this operating manual due to special designs, additional options specified on ordering and the latest technical/mechanical modifications.

This manual contains illustrations to aid general understanding. These may differ from the actual device as supplied.

## General Information

### 1.2 Explanation of Special Notices

#### 1.2.1 Warning Notices

Warning notices in this manual are indicated by a coloured bar and begin with a signal word that signifies the degree of the hazard.

##### **WARNING**

The signal word "WARNING" indicates a potentially dangerous situation that may result in severe or even fatal injuries if not avoided.

##### **CAUTION**

The signal word "CAUTION" indicates a potentially dangerous situation that may result in minor injuries if not avoided.

#### 1.2.2 Other Notices

##### **ATTENTION**

The word "ATTENTION" on a blue bar indicates a situation that may result in significant damage to property if not avoided.

##### **INFORMATION**

Texts located below a grey bar bearing the notice "INFORMATION" provide useful tips and recommendations for ensuring efficient, fault-free operation of the device.

### 1.3 Device Identification (Standard Identification Plate)

The identification plate is designed to allow clear identification of the device. It contains the following information:



- Manufacturer name
- Designation = Category of device
- Type = Device type (name)
- S/N = Serial number
- Year = Year of manufacture
- Mains = Nominal voltage and frequency
- Current = Current consumption
- Manufacturer address
- CE marking

### 1.4 Declaration of Conformity

The device is in compliance with the essential requirements of the following Directives:

- Directive 2006/42/EC on Machinery
- EMC Directive 2014/30/EU

The Declaration of Conformity according to Directive 2006/42/EC on Machinery, annex II 1 A is attached to the operating manual, refer to chapter "EC-Declaration of Conformity".

### 1.5 Customer Service and Services

Our Customer Service is at your disposal for technical advice and specialist enquiries. For contact information, see page 2.

Due to their familiarity with the potential applications of the device, the Customer Service team is able to provide information on whether the device can be used for a specific application or modified to handle the planned process.

Furthermore, our colleagues are always interested in new information and experiences resulting from user's applications for the device that may be valuable for the continued development of our products.

## Safety and Responsibility

## 2 Safety and Responsibility

This chapter describes general considerations relating to user safety that must be taken into account when working with the device.

In the remaining chapters, warning notices are used only to highlight particular hazards directly arising from the actions being described in the section in question.



**It is essential to read the operating manual carefully – especially this chapter and the warning notices in the text – and to follow the instructions therein.**

This chapter also refers to areas that are the responsibility of the provider due to certain risks arising from particular applications for which the device is used deliberately and with full awareness of the associated risks.

### 2.1 Intended Use, Incorrect Use and Misuse

**The bench-top bioreactor Minifors 2 from INFORS HT is designed especially for running bio processes with microorganisms or animal cells for research and development in a biotechnology laboratory.**

The device is designed and constructed exclusively for the intended use described above.

Intended use also includes following all the instructions in this operating manual, especially those relating to:

- The installation site
- User qualifications
- Correct operation and maintenance
- The use of undamaged tubing and glass vessels

Any failure to observe the requirements specified in this manual shall be deemed incorrect use.

Any use of the device outside the scope of the intended use as described above shall be deemed misuse.

This also applies to applications for which the device is not designed, such as the use or production of explosive gases, which is not permitted because the device is not explosion-proof.

## Safety and Responsibility

For use for special applications not covered by conventional, intended use, the device must be modified and certified accordingly by the manufacturer.

Any use of the device outside of a biotechnology laboratory, i.e. in any environment in which the conditions required for the safety of the users cannot be fulfilled or cannot be fulfilled to their full extent, shall also be deemed misuse.

## 2.2 Qualified Personnel

Due to the complexity of the device and the potential risks arising from its operation, the device may only be used by qualified, specialist personnel.

### 2.2.1 Provider

The term "provider" applies to all persons who are responsible for making the device and the necessary infrastructure available. These persons may also be included in the group of people known as "users", though this is not always the case.

Irrespective of whether a provider is a member of the company's board of management or a supervisor, they bear a special level of responsibility with regard to the processes and the qualification and safety of the users.

### 2.2.2 User

#### General

The term "user" applies to all persons who come into contact with the device in any way and perform work on or with it. This primarily applies to the following activities, which can be performed by the manufacturer's own specialists or a variety of other persons (it is not always possible to distinguish clearly between the different types of person):

- Assembly, installation and commissioning
- Definition and preparation of the process
- Operation
- Troubleshooting and remedying of faults
- Maintenance and cleaning (autoclaving, if necessary)
- Service work and repairs
- Disassembly, disposal and recycling

## Safety and Responsibility

### Qualified personnel

On account of their specific education, training and – in many cases – experience, the qualified personnel required for this work are able to recognise risks and respond accordingly to potential hazards.

The qualified personnel (either internal or external) who cannot be categorised under the separate “operators” group are made up of the following groups of persons:

- Electricians (electrical engineers)
- Decontamination specialists
- Repair specialists
- Specialists in disassembly and (environmentally friendly) disposal
- Recycling specialists

### 2.2.3 Operator

The “operators” are a specific sub-group of users distinguished by the fact that they work with the device. They are the true target audience for this operating manual.

### Qualified technicians

Only technicians who have been trained for working in a biotechnology laboratory can be considered for the role of operator. These include:

- Process technicians in the fields of biotechnology and chemistry
- Biotechnologists (biotechnicians)
- Chemists with a specialisation in biochemistry; chemists in the field of organic chemistry or biochemistry
- Life scientists (biologists) with special education in cytology, bacteriology, molecular biology, genetics, etc.
- Lab assistants (lab technicians) from various fields

In order to be classed as a “sufficiently qualified technician” for the operation of the device, the persons in question must have received thorough training and have read and understood the operating manual.

The operator must be informed in a training session provided by the provider of the tasks delegated to the operator and the potential risks of improper conduct. Tasks that go beyond the scope of operation under normal conditions may only be performed by the operator if

## Safety and Responsibility

this is specified in the manual and the provider has explicitly entrusted said tasks to the operator.

### Technicians in training

Persons in this group who are undergoing training or apprenticeships are only permitted to use the device under supervision and in accordance with the instructions of a trained and qualified technician.

## 2.3 Unauthorised Persons

The term “unauthorised persons” applies to all persons who can access the work area but are not qualified to use the device in accordance with the aforementioned requirements.

Unauthorised persons are not permitted to operate the device or use it in any other way.

## 2.4 Responsibility of the Provider

The device is used for industrial and scientific purposes. As such, the provider of the device is individually liable with regard to the legal requirements relating to occupational health and safety in a biotechnology laboratory. In particular:

- The provider is responsible for ensuring that the work and environmental regulations applicable in a biotechnology laboratory are observed.
- The provider must ensure that the device remains in safe and proper working condition throughout its entire term of use.
- The provider must ensure that all safety equipment is fully functional and is not disabled.
- The provider must ensure that the device is only worked on by qualified users, and that said users receive sufficient training.
- The provider must ensure that the protective equipment required for working with the device is provided and worn.
- The provider must ensure that this operating manual remains in the immediate vicinity of the device throughout its entire term of use.

## Safety and Responsibility

### 2.5 General Hazards

This chapter covers general hazards and residual risks that are always present when using the device in accordance with normal, intended use.

The following notices are general in nature. As such, with a few exceptions they are not repeated in the remaining sections.

#### 2.5.1 Electrical Current



The device runs on electrical power. There is an immediate risk of fatal injury if contact is made with live parts.

The following points must be observed in order to avoid the risk of fatal injury:

- In case of damage to insulation, disconnect the device from the power supply immediately and arrange for it to be repaired.
- Disconnect the device from the power supply before commencing any work on the electrical system.
- Always use qualified electricians for any work on the electrical system.
- Keep moisture away from live parts. It may lead to a short circuit.

#### 2.5.2 Unauthorised Spare Parts and Accessories



Incorrect or imitated spare parts and accessories as well as spare parts or accessories that have not been authorised by the manufacturer represent a significant safety risk. As such, we recommend procuring all spare parts and accessories from an authorised dealer or directly from the manufacturer. For the contact details of the manufacturer's representatives, see page 2.

### 2.6 Particular Hazards

This chapter covers particular hazards and residual risks that may arise when using the device for special applications in accordance with normal, intended use.

Since the use of the device for such applications is deliberate, it is the responsibility of the operators and the provider to ensure that all

## Safety and Responsibility

personnel are protected from potential damage to health. The provider is responsible for ensuring that the appropriate protective equipment for such applications is provided, and that the necessary infrastructure is in place.

### 2.6.1 Hot Surfaces



For processes that are carried out with temperatures over 55 °C, there is a danger of burns on hot surfaces.

Since the device is intended for applications at high temperatures, it is the responsibility of the users to ensure that they have sufficient protection.

The thermal block and its adapter get hot during operation. There is a risk of burns, if touched.

#### Version for microorganisms

The motor gets hot during operation. There is a risk of burns if it is touched.

### 2.6.2 Dangerous Gases



The use or production of dangerous gases i.e. toxic or asphyxiant gases entails a significant health risk, especially in enclosed spaces.

In order to prevent high emissions of dangerous gases, the following measures must be taken:

- The gas connections on the device must be checked before any cultivation processes using dangerous gases are initiated.
- The gaskets on the device must be checked at regular intervals and replaced if necessary.
- Siphon off exit gas safely.

### 2.6.3 Flammable or Explosive Substances



The use or production of flammable or explosive substances is not covered under "intended use" of the device, as the device is not explosion-proof.

If the provider intends to use the device for such purposes, he must check its suitability for the planned application with the responsible local authorities.

## Safety and Responsibility

### 2.6.4 Corrosive or Toxic Substances



The use or production of corrosive or toxic substances entails a significant health risk. As such, special measures must be taken to protect the users for such applications.

Since the device is used deliberately for such applications, it is the responsibility of the users to ensure that they have sufficient protection.

### 2.6.5 Bioactive Substances or Pathogenic Organisms



The use or production of bioactive substances, pathogenic organisms or genetically modified cultures entails a significant health risk. As such, special measures must be taken to protect the users for such applications.

Since the device is used deliberately for such applications, it is the responsibility of the users to ensure that they have sufficient protection.

### 2.6.6 Overpressure or Vacuum



Glass vessels may break or shatter when subjected to overpressure or vacuums.

## 2.7 Warning Symbols on the Device

The following warning symbols (stickers) are attached to the device:

#### Position

- Thermal block adapter
- Motor (version for microorganisms)



#### WARNING

Illegible or missing warning symbols on the device will lead to the user being exposed to risks that the warning symbols in question were designed to make him or her aware of.

It is the provider's responsibility to ensure that all the stickers with warning symbols on the device are always intact.

## 2.8 Declaration of Decontamination

When returning the device for repair, disassembly or disposal, it is required for the safety of all parties involved and because of legal provisions that a lawful declaration of decontamination is present.

The following must be observed if this is the case:

- The device, the component part or accessory must be entirely decontaminated before sending to the manufacturer
- The provider is therefore required to completely and truthfully fill out a declaration of decontamination, and have it signed by the person responsible.
- **The declaration of decontamination must be affixed on the outer packaging in which the device is sent back.**
- These forms can be obtained from the licensed dealer or the manufacturer. See address on page 2.

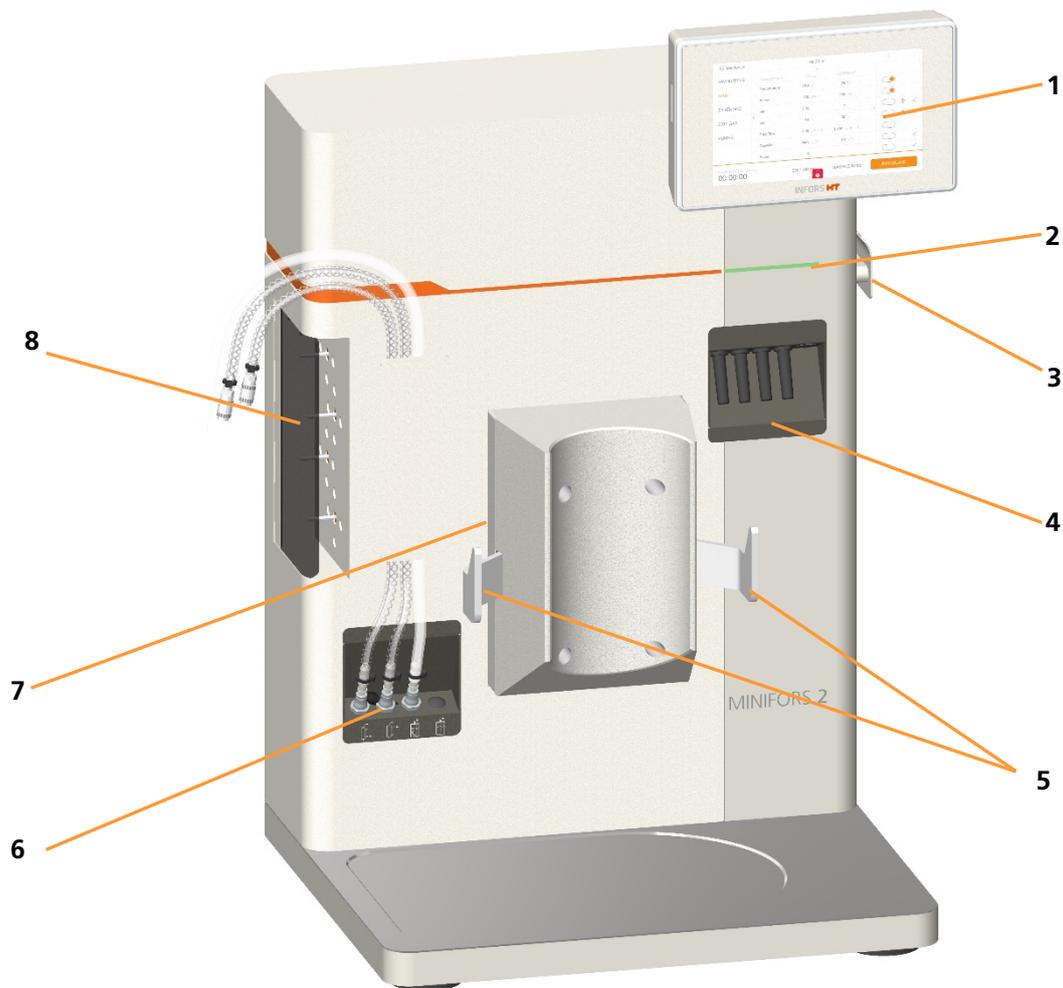
### Important notice

If the return shipment is not accompanied by a signed and complete declaration of decontamination or it is not affixed to the outer packaging, the shipment will be returned unopened to the sender at their expense (see also T&C).

## Setup and Function

### 3 Setup and Function

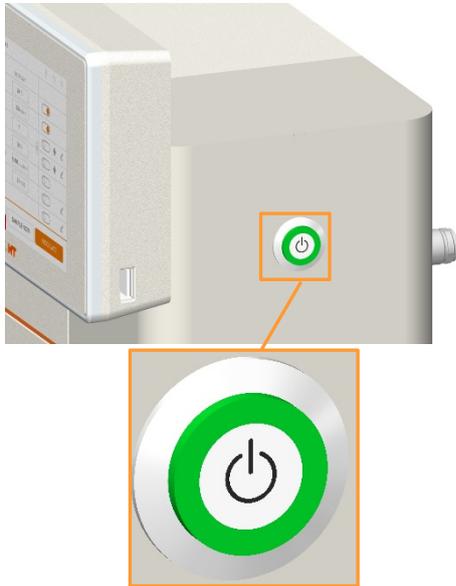
#### 3.1 Basic Unit



- |   |                         |   |                                             |
|---|-------------------------|---|---------------------------------------------|
| 1 | Operating panel         | 5 | Hooks for vessel holder                     |
| 2 | LED signal strip        | 6 | Connections for gassing and exit gas cooler |
| 3 | Power switch            | 7 | Thermal block and adapter                   |
| 4 | Connections for sensors | 8 | Pumps                                       |

All of the measurement and control technology is built into the basic unit. The basic unit is equipped as standard with a thermal block plus adapter for regulating the temperature of the culture vessel, four pumps for adding reagents and feed solution, and the operating panel.

### 3.1.1 Power Switch



The power switch is a pressure switch which is located on the right-hand side on the basic unit. It lights up green as soon as the device is switched on. In addition to normal switching on and off, the power switch also serves as an emergency switch.

#### **i** INFORMATION

In the event of an emergency shutdown via the power switch during a running Batch (process), all settings are saved. After switching on via the power switch, the Batch continues with the same settings as before the emergency shutdown. This is also the case if the Batch is controlled via eve®, the platform software for bioprocesses.

### 3.1.2 LED Strip – Status Indicator



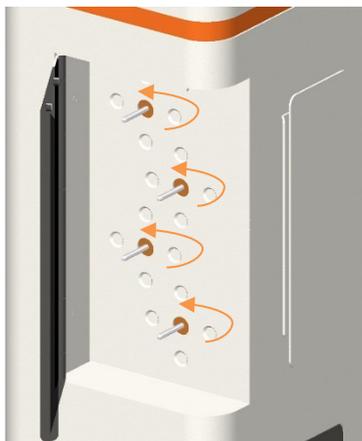
The LED strip is located on the front of the basic unit and indicates the following:

- Green steady light: the device is functioning as normal. (The led strip lights up green as soon as the device is switched on).
- Green blinking light: one or several parameter alarm(s) has/have occurred. For more details, see main chapter "Operation", chapter "Parameter Alarms".
- Red blinking light: one or several device error(s) has/have occurred. For more details, refer to the main chapter "Interferences".

## Setup and Function

### 3.1.3 Pumps

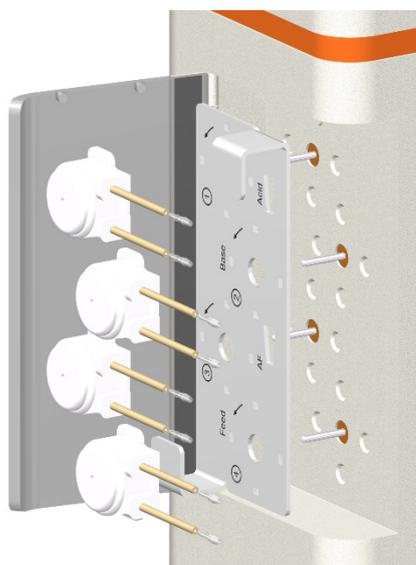
Reagents and feed solution are supplied via four peristaltic pumps. The pumps are driven by stepper motors.



The pump drive shafts are located on the left-hand side of the basic unit. The drive shafts' direction of rotation is set as standard to anti-clockwise for "filling"; see marking on mounting plate. The pumps can be configured individually using the operating panel, and thus each set to digital or analogue operating mode as required:

- Digital = OFF/ON operation with fixed speed
- Analogue = continuous operation with variable speed.

A hinged Plexiglas cover acts as a guard during operation.



The autoclavable pump heads are plugged into a mounting plate. (Depicted separately here in order to show the marking under the pumps.) This is numbered 1 to 4 from top to bottom, and labelled to indicate the standard factory settings:

- Pump 1: *Acid* (digital)  
Alternative setting: *Feed* (analogue)
- Pump 2: *Base* (digital)  
Alternative setting: *Feed* (analogue)
- Pump 3: *AF* (antifoam, digital)  
Alternative setting: *Level* (digital) or *Feed* (analogue)
- Pump 4: *Feed* (analogue)  
Alternative setting: *Balance* or *Dose* (analogue)

For more information on possible pump settings, see main chapter "Operation", chapter "Parameter Group PUMPS".

The pump heads and the mounting plate can be simply pushed onto or pulled off the drive shafts.

### 3.1.4 Identification Plate

The identification plate is located at the side of the basic unit.

The data provided on the identification plate is specified in the main chapter "General Information", chapter "Device Identification".

### 3.1.5 Mains Connection and Device Fuses



The mains connection is located at the bottom left of the back of the basic unit.

The device is protected against excessive current consumption by two fuses. The device fuses are located directly above the mains connection.

The country-specific power cable required for connection to the power supply is included in the scope of delivery. If the power cable is defective, replace it with a power cable of the same type.

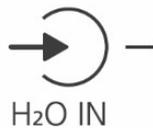
Before connecting the device, make sure that the voltage values of the device correspond to the local mains voltage. The mains connection must be easily accessible at all times so that the device can be disconnected from the power supply quickly in case of an emergency.



## Setup and Function

### 3.1.6 Water Connections

The water connections are located on the rear of the basic unit, at the bottom right. They are marked with the following symbols:



- **H<sub>2</sub>O IN:** water inlet



- **H<sub>2</sub>O OUT:** water outlet

### 3.1.7 Gas Connections

The connections for the gas supply are located on the rear of the basic unit, at the bottom right, above the water connections.

They are marked with the following symbols:



- **CO<sub>2</sub> IN:** Inlet CO<sub>2</sub> (carbon dioxide)



- **N<sub>2</sub> IN:** Inlet N<sub>2</sub> (nitrogen)



#### INFORMATION

These two gas supply connections are closed with blanking plugs. They are used in the device version for cell cultures.



- **O<sub>2</sub>/GAS 2 IN:** Inlet O<sub>2</sub> (oxygen) / or 2nd gas



- **AIR IN:** Inlet air

### 3.1.8 Signal Connections

The following signal connections with the corresponding symbols and labelling are located on the left rear side of the basic unit:



- **ANALOG I/O:** analogue input/output for connection of external devices.

It has a connector with PUSH IN spring connection.

Analog Output 2	■	■	GND
Analog Output 1	■	■	GND
Analog Input 2	■	■	GND
Analog Input 1	■	■	GND

Pin assignment of the connector:

- Output 1+2: setpoint entry & measured value output
- Input 1+2: measured value output only



- **SERVICE:** 9-pin RS232 for connecting a diagnostic computer for maintenance.



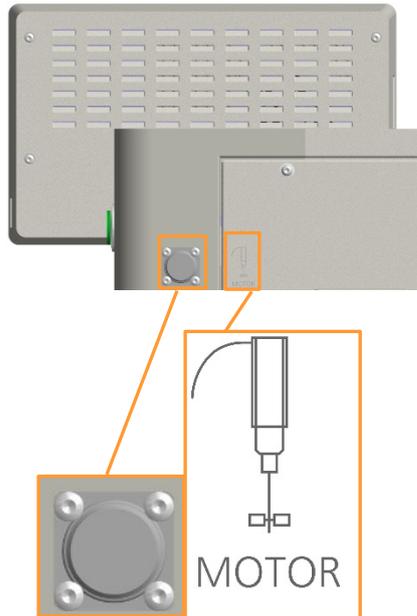
- **BALANCE:** 9-pin RS232 for connecting a balance.



- **LAN:** port for connecting a network cable.

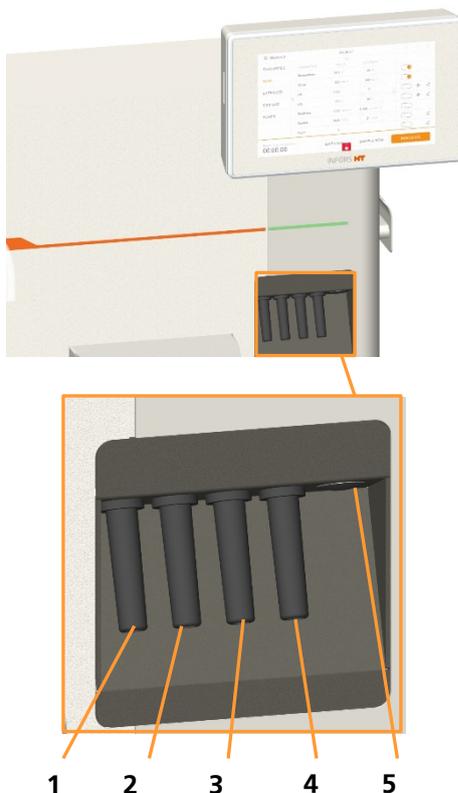
## Setup and Function

### 3.1.9 Motor Cable Connection



The connection for the motor cable is located on the rear of the basic unit at the top left, and marked with a corresponding symbol.

### 3.1.10 Connections for Sensors (Sensor Cables)



The basic unit is equipped and configured by default for measurement of temperature, pH, pO<sub>2</sub> and for foam detection (“antifoam”). This means, the temperature sensor (Pt100) and the cables for connecting the pH, pO<sub>2</sub> and antifoam sensors are always present. The appropriate sensors are included in the standard package.

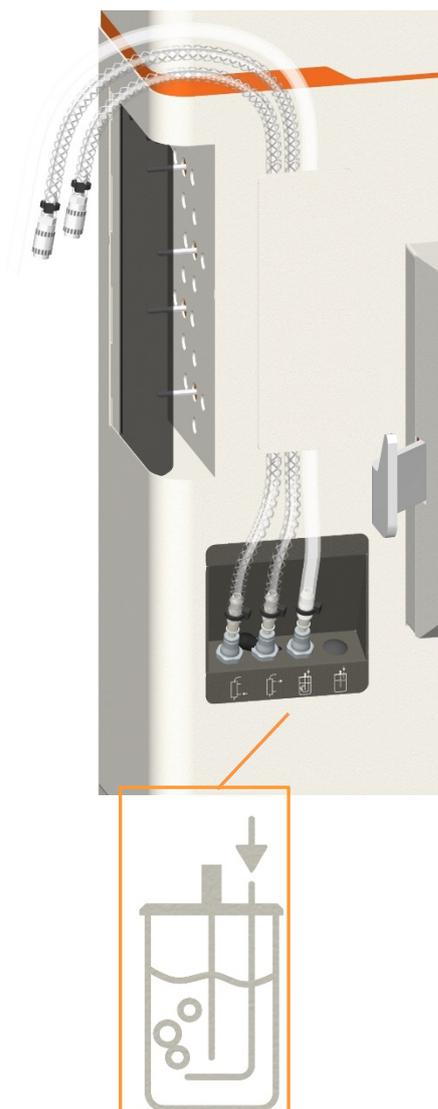
- 1 pO<sub>2</sub>
- 2 Temperature (Pt100)
- 3 Antifoam
- 4 pH
- 5 Spare connection for turbidity measurement sensor (option)

### 3.1.11 Gassing Connections (Sparger & Head Space)

The connections for gassing are located on the front of the basic unit, at the bottom left. They are marked with a corresponding symbol.

#### Version for microorganisms

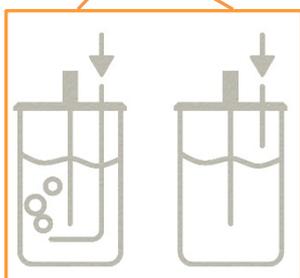
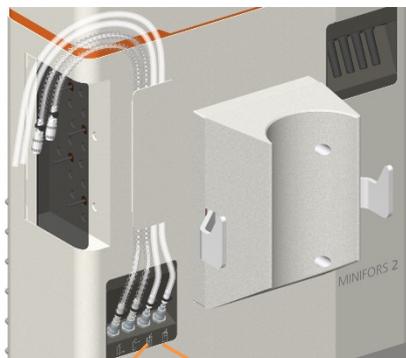
The hose used to connect the sparger for gassing is connected to the basic unit's gassing connection ex-factory.



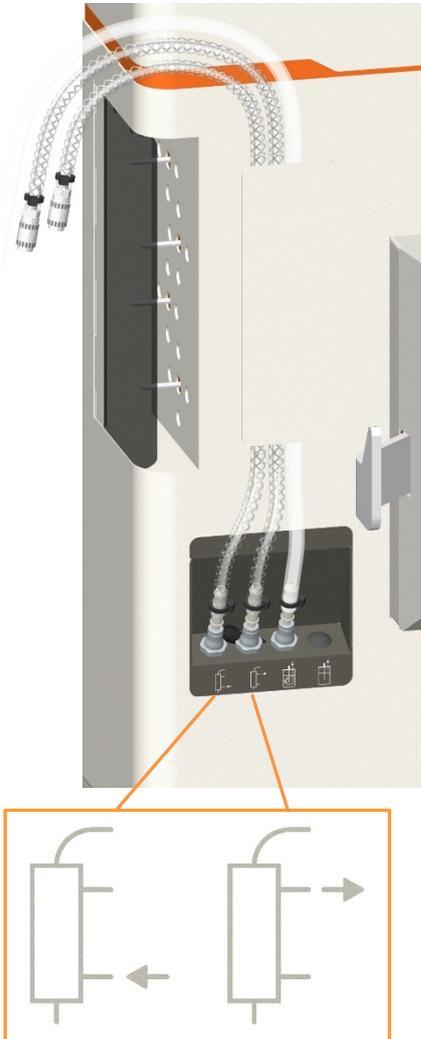
## Setup and Function

### Version for cell culture

The hoses used to connect the sparger & the addition port adapter for head space gassing are connected to the basic unit's gassing connection ex-factory.



### 3.1.12 Connections and Water Flow Control Valve for the Exit Gas Cooler



The water connections for the exit gas cooler are located on the front of the basic unit, at the bottom left.

The water supply and return hoses for the exit gas cooler are connected to the basic unit in the factory. The rapid couplings at both ends of the hoses are used to connect them to the exit gas cooler.

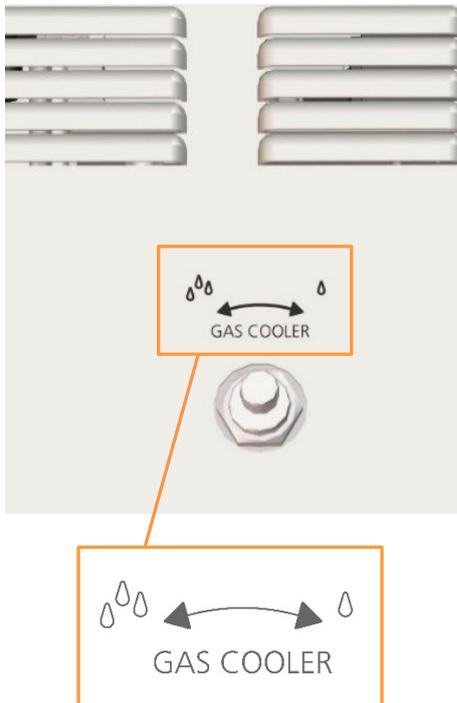
**i** INFORMATION

Due to the different hose lengths, it is not possible to connect them to the exit gas cooler incorrectly.

The water connections are marked with the corresponding symbols:

- To the left: exit gas cooler water inlet
- To the right: exit gas cooler water outlet

## Setup and Function



The water flow control valve is located on the rear of the basic unit, labelled with **GAS COOLER** and marked with a corresponding symbol.

The control valve is set at the factory. If necessary, the water flow rate can be set manually using the control valve:

- Turn anti-clockwise to increase the water flow rate
- Turn clockwise to reduce the flow rate

The valve can be fixed in the required position using a lock nut.

### 3.2 Operating Panel



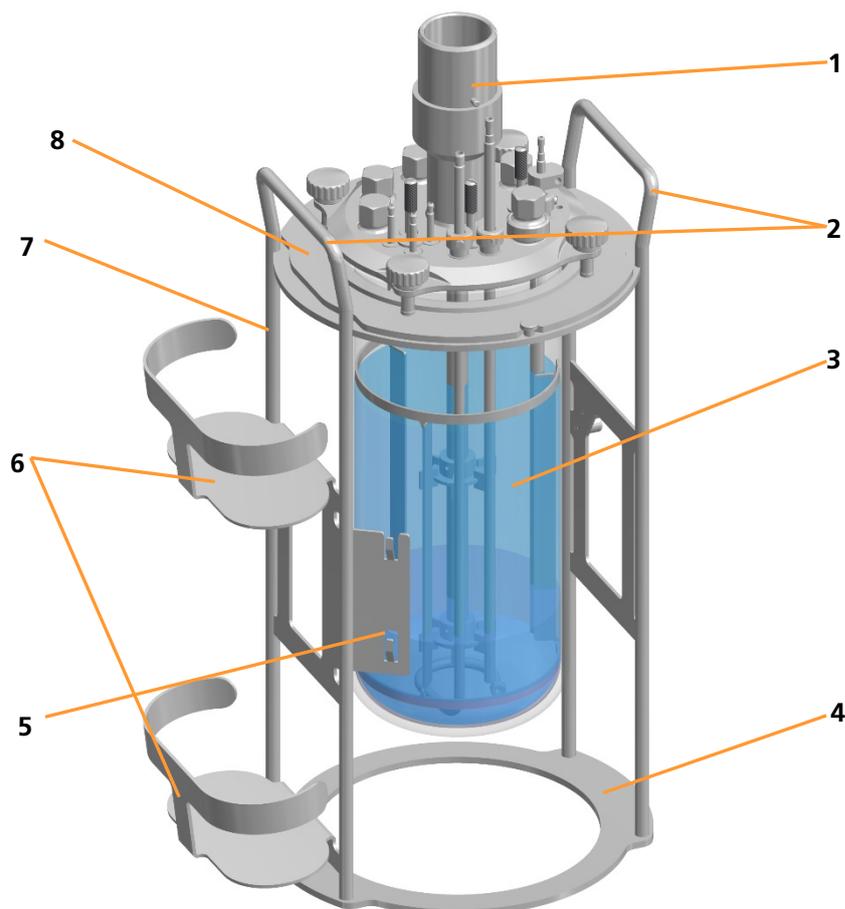
The operating panel on the top right of the basic unit has a 7" TFT touch screen.

- On the right-hand side of the panel is a USB port.
- On the left-hand side is a slot for an SD card

The operating panel is switched on using the power switch. For a detailed description of how to operate it, refer to main chapter "Operation".

### 3.3 Culture Vessel

The culture vessel comprises a glass vessel, the top plate with the standard fittings (which vary based on vessel size) and the vessel holder with handles. The vessel is made of borosilicate glass.



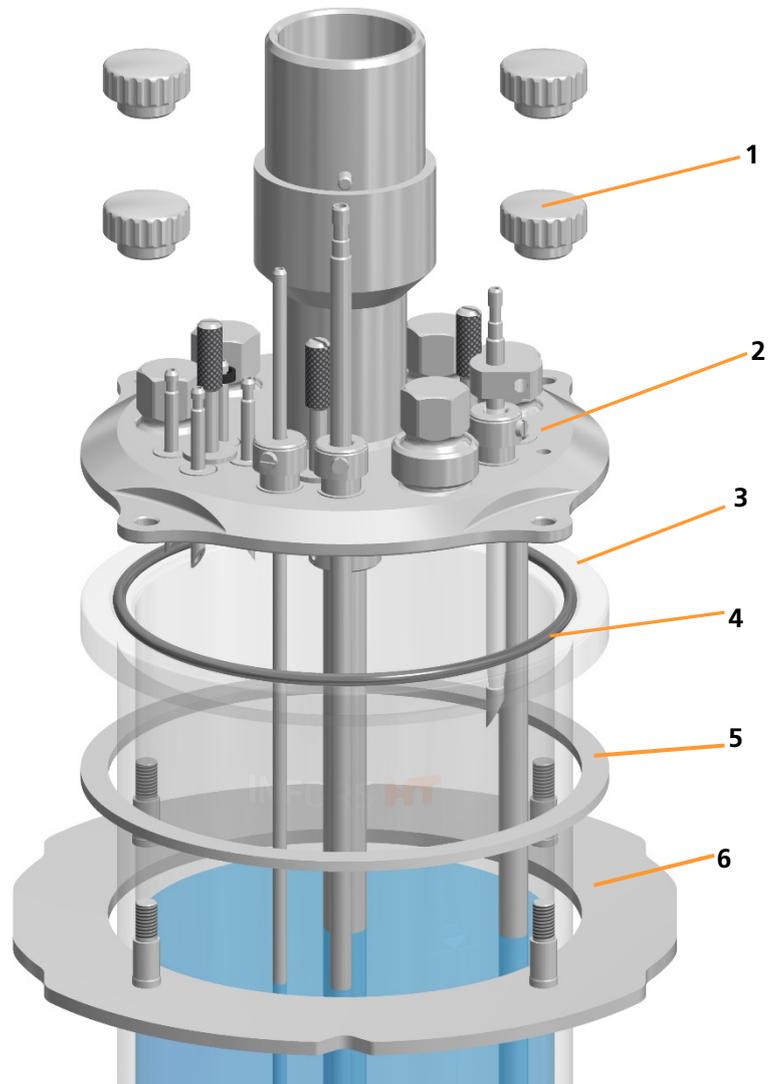
- |   |                      |   |                       |
|---|----------------------|---|-----------------------|
| 1 | Motor coupling       | 5 | Pump holder           |
| 2 | Vessel holder handle | 6 | Reagent bottle holder |
| 3 | Glass vessel         | 7 | Vessel holder         |
| 4 | Vessel holder stand  | 8 | Top plate             |

The illustration shows a culture vessel for microorganisms with a total volume of 1.5 L and a nominal diameter of 90 mm. There are three vessel sizes available, each with a matching top plate.

The vessel holder has two handles on the side, which are used when emptying and cleaning the vessel or transporting it to the autoclave.

## Setup and Function

### 3.3.1 Top Plate



- 1 Knurled nut (x4)
- 2 Top plate
- 3 Vessel

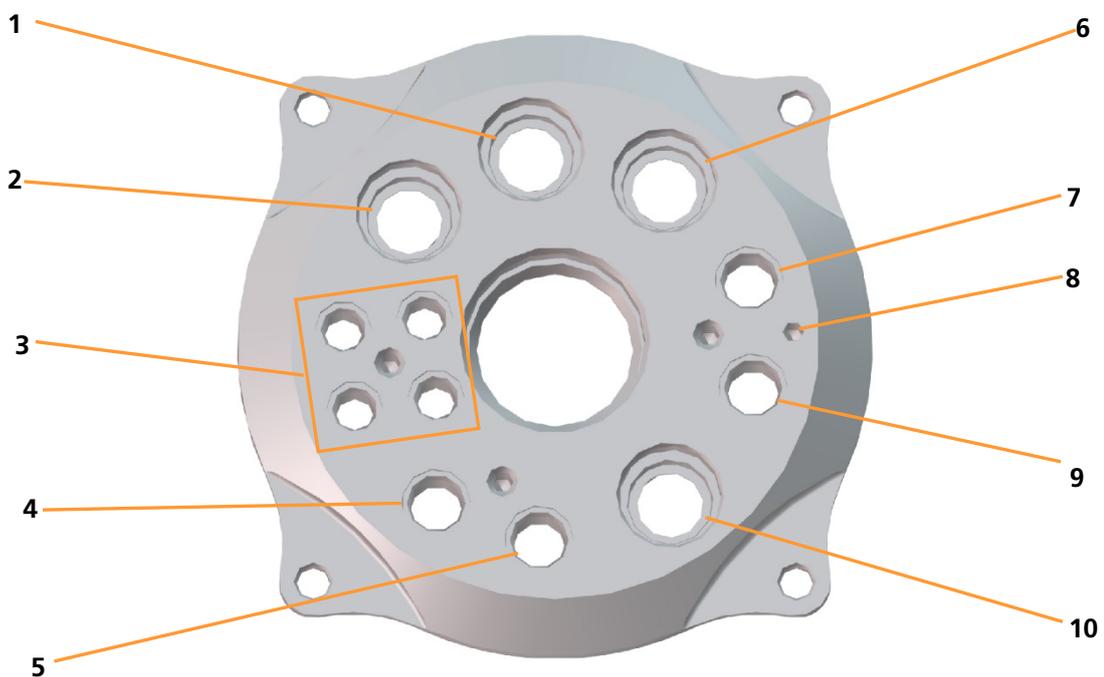
- 4 O-ring
- 5 Damping ring (spacer ring)
- 6 Flange

The top plate is attached to the vessel using four knurled nuts and a flange. The knurled nuts also hold the vessel in place in the vessel holder. An O-ring is used to seal the top plate. A spacer ring is used to prevent the top plate from exerting pressure on the rim of the vessel.

### 3.3.2 Ports in the Vessel Top Plate and their Configuration

The vessel top plate has different ports of different sizes to mount the different components such as sparger, blanking plugs, sensors etc. The number of ports in the top plate and its configuration depends on the nominal diameter (DN) = inner diameter) of the culture vessel.

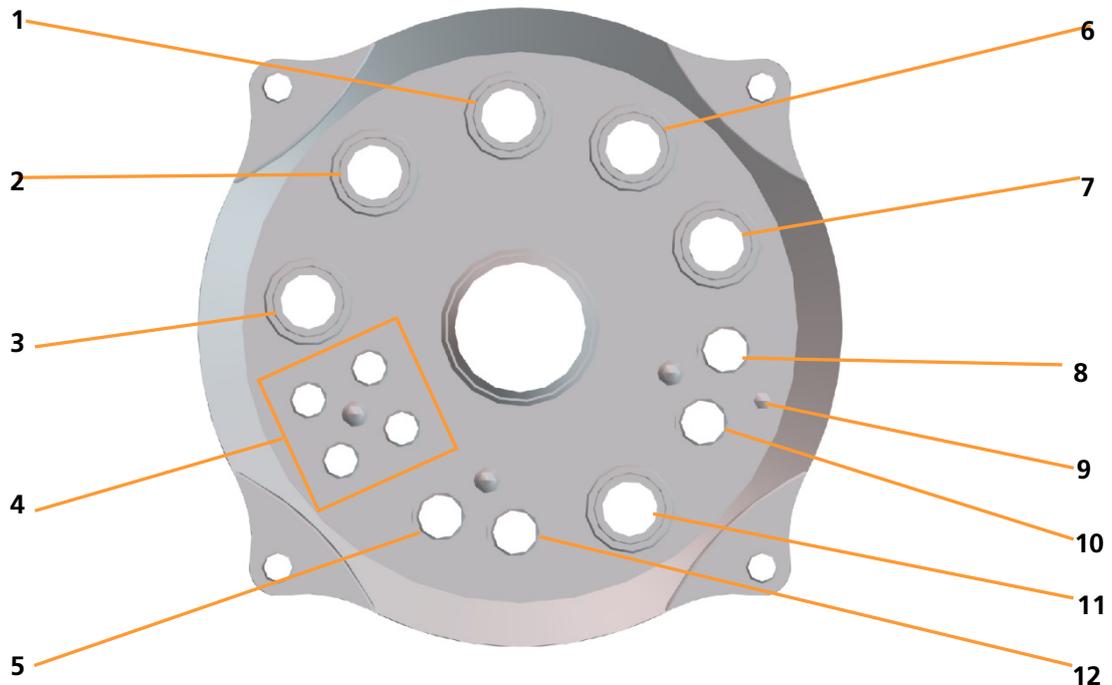
#### 3.3.3 Vessel Top Plate, DN 90



- |   |                                           |    |                                                      |
|---|-------------------------------------------|----|------------------------------------------------------|
| 1 | Ø 12 mm Pg13.5: pH sensor                 | 7  | Ø 10 mm: immersion pocket temperature sensor (Pt100) |
| 2 | Ø 12 mm Pg13.5: exit gas cooler           | 8  | Ground connection antifoam sensor                    |
| 3 | Ø 7.5 mm: addition port adapter, 4 pieces | 9  | Ø 10 mm: antifoam sensor                             |
| 4 | Ø 10 mm: sparger                          | 10 | Ø 12 mm Pg13: inoculation                            |
| 5 | Ø 10 mm: dip tube sampling                |    |                                                      |
| 6 | Ø 12 mm Pg13.5: pO <sub>2</sub> sensor    |    |                                                      |

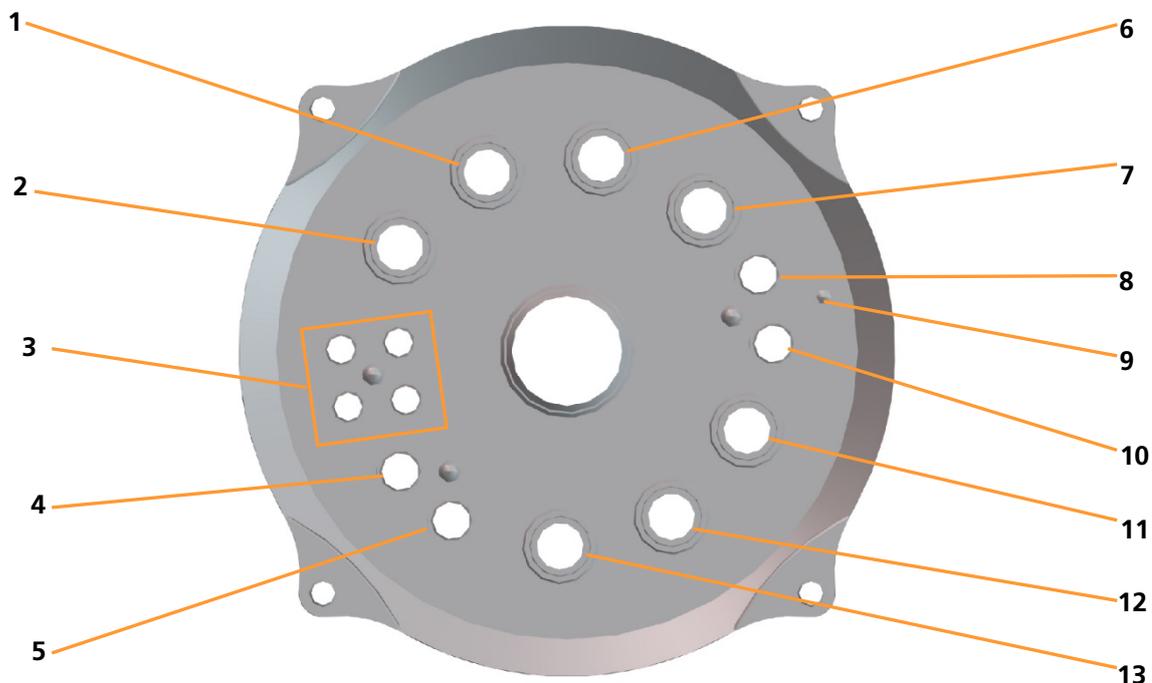
## Setup and Function

### 3.3.4 Vessel Top Plate, DN 115



- |   |                                           |    |                                                      |
|---|-------------------------------------------|----|------------------------------------------------------|
| 1 | Ø 12 mm Pg13.5: pH sensor                 | 8  | Ø 10 mm: immersion pocket temperature sensor (Pt100) |
| 2 | Ø 12 mm Pg13.5: exit gas cooler           | 9  | Ground connection antifoam sensor                    |
| 3 | Ø 12 mm Pg13.5: additional sensor         | 10 | Ø 10 mm: antifoam sensor                             |
| 4 | Ø 7.5 mm: addition port adapter, 4 pieces | 11 | Ø 12 mm Pg13.5: inoculation                          |
| 5 | Ø 10 mm: sparger                          | 12 | Ø 10 mm: dip tube sampling                           |
| 6 | Ø 12 mm Pg13.5: pO <sub>2</sub> sensor    |    |                                                      |
| 7 | Ø 12 mm Pg13.5: additional sensor         |    |                                                      |

### 3.3.5 Vessel Top Plate, DN 145

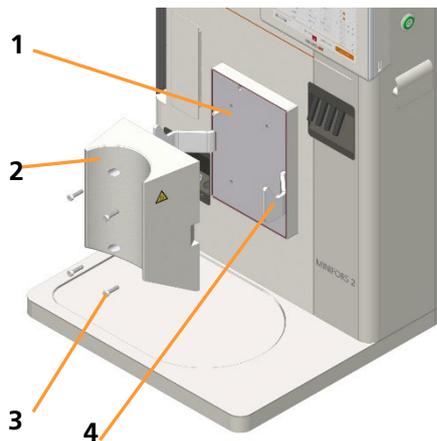


- |   |                                           |    |                                                      |
|---|-------------------------------------------|----|------------------------------------------------------|
| 1 | Ø 12 mm Pg13.5: exit gas cooler           | 8  | Ø 10 mm: immersion pocket temperature sensor (Pt100) |
| 2 | Ø 12 mm Pg13.5: additional sensor         | 9  | Ground connection antifoam sensor                    |
| 3 | Ø 7.5 mm: addition port adapter, 4 pieces | 10 | Ø 10 mm: antifoam sensor                             |
| 4 | Ø 10 mm: sparger                          | 11 | Ø 12 mm Pg13.5: additional sensor                    |
| 5 | Ø 10 mm: dip tube sampling                | 12 | Ø 12 mm Pg13.5: additional sensor                    |
| 6 | Ø 12 mm Pg13.5: pH sensor                 | 13 | Ø 12 mm Pg13.5: inoculation                          |
| 7 | Ø 12 mm Pg13.5: pO <sub>2</sub> sensor    |    |                                                      |

## Setup and Function

### 3.4 Temperature Control System

The temperature (heating and cooling) is controlled using a thermal block and its adapter.

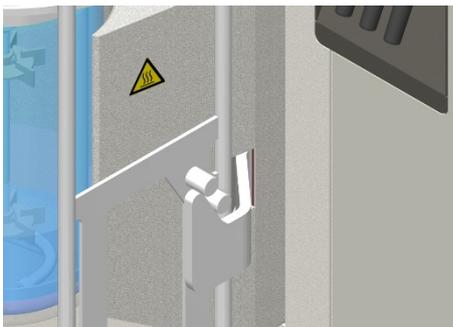


- 1 Thermal block
- 2 Thermal block adapter
- 3 Fastening screw (Allen screw, 4 pieces)
- 4 Hook, 2 pieces

There is a thermal block adapter for each vessel size. The thermal block adapters are screwed onto the thermal block.

The temperature in the culture vessel is measured using a platinum resistor temperature sensor (Pt100). The temperature is transmitted from the thermal block to the adapter and from the adapter to the culture vessel by means of heat exchange.

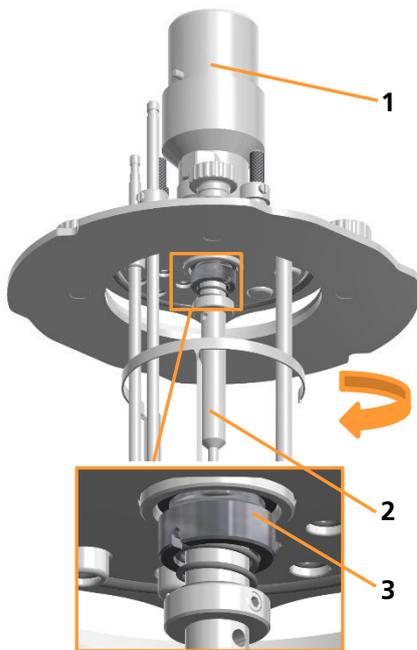
The thermal block is heated electrically using heating cartridges. It is cooled by water flowing through it.



The two hooks on the thermal block hold the culture vessel in place on the basic unit. In order to ensure optimum heat transmission, the two hooks also pull the culture vessel right up against the thermal block.

### 3.5 Stirrer

The stirrer shaft is driven from above and turns anti-clockwise (left when viewing vessel from above).



- 1 Drive hub
- 2 Stirrer shaft
- 3 Mechanical seal

The stirrer shaft is sealed using a mechanical seal.

#### 3.5.1 Motor

##### Version for microorganisms

A brushless gear motor with a mechanical coupling is used as standard. Depending on the size of the vessel, two motors with different power levels are used; see main chapter "Technical data" chapter "Specifications", "Stirrer".

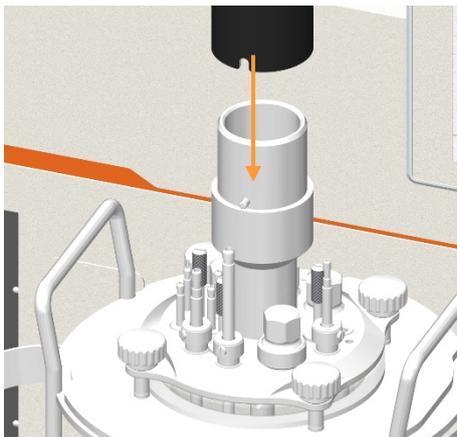


- Left: Small motor for culture vessels DN 90
- Right: Large motor for culture vessels DN 115 and 145

## Setup and Function

### Version for cell culture

The same brushless gear motor with mechanical coupling is used for all vessel sizes.

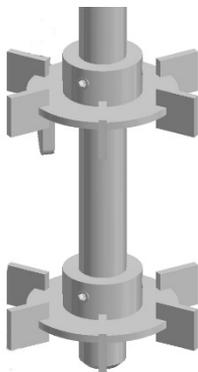


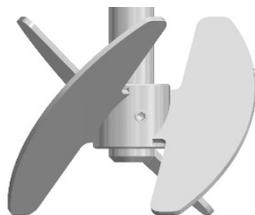
The motor is connected by pushing it onto the drive hub on the top plate.

### 3.5.2 Impellers

#### Version for microorganisms

Two Rushton impellers are attached to the stirrer shaft by means of grub screws.



**Version for cell culture**

One pitched blade impeller is attached to the stirrer shaft by means of grub screws.

### 3.6 Gassing System

The following gases can be used:

**Version for microorganisms**

- Air
- Oxygen (O<sub>2</sub>) or Nitrogen (N<sub>2</sub>)

The basic unit is equipped and configured with two mass flow controllers for controlling the gas flow and, if necessary, the gas mixture. If oxygen or nitrogen are used in addition to air, the gases are mixed before being fed into the culture vessel. Both the gas flow rate(s) and the composition of the gas mixture (where applicable) are set using the operating panel.

**Version for cell culture**

- Air
- Oxygen (O<sub>2</sub>)
- Nitrogen (N<sub>2</sub>)
- Carbon dioxide (CO<sub>2</sub>)

The basic unit is equipped and configured with five mass flow controllers for controlling the gas flow and the gas mixture.

Air, oxygen and nitrogen are used for sparger gassing. Additionally, air can be used for head space gassing. CO<sub>2</sub> can be used instead of liquid acid for pH control and is either added via sparger or head space. The gases are mixed before being fed into the culture vessel. Both the gas flow rate(s) and the composition of the gas mixture are set using the operating panel.

## Setup and Function

### 3.6.1 Gas Entry

#### Version for microorganisms

A hose line leads the gas or gas mixture from the gassing connection on the basic unit to the culture vessel, via a sterile filter. The gas is fed directly into the medium via the sparger (sparger gassing).

#### Version for cell culture

Two hose lines lead the gas or gas mixture from the gassing connections (sparger and head space) on the basic unit to the culture vessel, via sterile filters. Gas(es) is/are fed directly into the medium via the sparger (sparger gassing). For head space gassing, the gas is led via addition port adapter into the head room of the culture vessel, i.e. above the culture medium.

### 3.6.2 Exit Gas

Even without active gassing, any cultivation can increase the pressure inside the vessel through heating or gas production. As such, an exit gas line is essential for all cultivation processes.

#### Siphoning off exit gas via the exit gas cooler

The exit gas cooler dries the exit gas through condensation, thus preventing the exit gas filter from becoming clogged with moisture. At the same time, it also prevents liquid loss in the culture medium.



#### INFORMATION

If heavy foaming is expected, a bottle of antifoam agent can be installed upstream of the exit gas filter as a foam trap.

The exit gas cooler is included in the standard package; for more details, see main chapter "Accessories", chapter "Exit Gas Cooler".

## 3.7 pH Control

The pH value in the medium is measured by the pH sensor and regulated by addition of reagents (acid, base). Addition of acid and base takes place via the two peristaltic pumps *Acid* and *Base*.

Reagent bottles are filled with acid and base which are connected to an addition port adapter in the vessel top plate and the two pumps by silicone hoses.

### Version for cell culture

CO<sub>2</sub> can be used instead of liquid acid for pH control and is either added via sparger or head space.

### 3.7.1 Measurement System

The measurement system for pH is equipped and configured for digital sensors either from the manufacturer HAMILTON or METTLER.

The appropriate pH sensor is included in the device package. pH sensors type Easyferm Plus ARC are preconfigured by the device manufacturer INFORS HT. Replacement sensors must be configured before use.

#### Variant METTLER

- Conventional pH sensor (potential measurement against reference) with built-in electronics
- Type: InPro325i, ISM

#### Variant HAMILTON

- Conventional pH sensor (potential measurement against reference) with built-in electronics
- Type: Easyferm Plus ARC

Details on technical data, use and maintenance of the pH sensors can be found in the separate documentation of the sensor manufacturer. Read and follow instructions stated in there.

#### Calibration

Calibration of the pH sensor is always carried out **BEFORE** autoclaving. This is done on the operating panel. For more details on this procedure refer to the main chapter "Operation", chapter "Calibrating the pH Sensor".

## Setup and Function



### INFORMATION

If the pH sensor has already been calibrated before connection to the system, the bioreactor will use this data and calibration on the operating panel is no longer necessary.

### Mounting

For culture vessels DN 90 and DN 145, pH sensors can be mounted directly into 12 mm/Pg13.5 ports. For culture vessels DN115, an electrode holder is used. For more details on the electrode holder, refer to the main chapter "Accessories", chapter "Electrode Holder".

## 3.8 pO<sub>2</sub> Control

The oxygen saturation of the (culture) medium is measured by the pO<sub>2</sub> sensor, and can be adjusted as follows:

### Increasing the pO<sub>2</sub>

The content of the oxygen dissolved in the medium (pO<sub>2</sub>) can be increased using the following methods:

- Increasing the stirrer speed
- Increasing the gas volume flow rate (air and/or oxygen)
- Increasing the oxygen content in the Gasmix

These approaches can also be combined

### pO<sub>2</sub> reduction

In anaerobic processes, the vessel can be gassed using nitrogen. This displaces the oxygen dissolved in the medium.

### 3.8.1 Measurement System

The measurement system for pO<sub>2</sub> is equipped and configured for digital sensors either from the manufacturer HAMILTON or METTLER.

The appropriate pO<sub>2</sub> sensor is included in the device package. The pO<sub>2</sub> sensors are pre-configured by the device manufacturer, INFORS HT. Replacement sensors must be configured before use.

## Setup and Function

### Variant METTLER

- pO<sub>2</sub> sensor with built-in opto-electronics
- Type: InPro6860i, ISM, choice of:
  - Classic, with Opto-Cap, straight
  - HD, with Opto-Cap angled, with "Anti-Bubble" technology low-noise measurement signal.

### Variant HAMILTON

- pO<sub>2</sub> sensor with built-in opto-electronics
- Type: Visiform DO ARC, choice of:
  - ODO-Cap H0, straight, standard applications
  - ODO-Cap H2, convex, more robust, slightly longer response time.

Details on technical data, use and maintenance of the pO<sub>2</sub> sensors can be found in the separate documentation of the sensor manufacturer. Read and follow instructions stated in there.

Generally speaking, the following applies: Unlike measurements such as pH, which are calibrated to absolute measurement values, the oxygen measurement is always calibrated to a relative reference point. For this purpose, the calibration is set to 100 % relative oxygen saturation, usually with air at max. stirring speed and maximum gas flow rate. The absolute concentration of dissolved oxygen in mmol/L may therefore vary at 100 % saturation, depending on the process.

Depending on the specifications defined by the user, the pO<sub>2</sub> sensor will be calibrated either before the vessel is filled with medium or afterwards, in the prepared medium. For more details on this procedure refer to main chapter "Operation", chapter "Calibrating the pO<sub>2</sub> Sensor".

### Mounting

For culture vessels DN 90 and DN145, pO<sub>2</sub> sensors can be mounted directly into 12 mm/Pg13.5 ports. For culture vessels DN 115, an electrode holder is used. For more details on the electrode holder, refer to main chapter "Accessories", chapter "Electrode Holder".

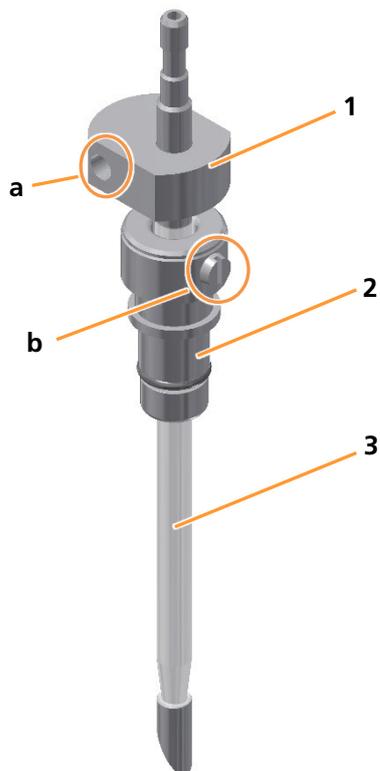
## Setup and Function

### 3.9 Antifoam Control

Foam hinders the exchange of gas between the medium and the gas phase in the head space. The exit gas filter can become clogged with foam, which causes a pressure build-up in the vessel. This can be prevented by adding antifoam agent.

The antifoam agent is kept in a reagent bottle that is connected to the antifoam sensor and the antifoam pump via a hose. The sensor also acts as a dosing needle. When the sensor comes in contact with foam, the antifoam pump is activated and antifoam agent is fed into the vessel via the dosing needle.

#### 3.9.1 Antifoam Sensor



Inside diameter	2 mm
Hose connection outside diameter	4 mm

A clamping adapter with a fixed O-ring is used to mount the sensor in the 10 mm port in the vessel top plate.

- 1 Sensor head with port for banana connector (a)
- 2 Clamping adapter with slotted-head screw (b)
- 3 Needle with transparent insulation

The antifoam sensor is equipped with two NON-autoclavable protective caps.

## 4 Options

The following options are available in addition to the equipment included in the scope of supply for the basic unit.

### 4.1 Turbidity Measurement

Turbidity measurement can be used to draw conclusions regarding the biomass concentration in the culture. To determine the turbidity in the culture two measurement systems are available:

#### Variant OPTEK

- Sensor (Single channel light absorption) with transmitter integrated in the basic unit
- Type: ASD12-N with two optical path lengths
  - Version for microorganisms: OPL05 for higher cell densities.
  - Version for cell culture: OPL10 for lower cell densities.
- Manufacturer: Optek
- Measures the absorption within a range of 0 to 4 CU

The ASD12-N sensors supply a non-linearised turbidity measurement for the culture. This can be linearised manually using the soft sensor in eve<sup>®</sup>, for example, to determine correlation with factors such as the biomass concentration or optical density.



#### INFORMATION

If the temperature of the sensor rises above 50 °C during operation in the medium, an automatic switch-off takes place. After the medium has cooled down, the measurement continues automatically.

#### Variant aquila biolabs

- Sensor (non-invasive scattered light measurement) with transmitter integrated in the basic unit (CGQ BioR gateway)
- Type: CGQ BioR with two LEDs / measurement modes:
  - Green: (521 nm) for low cell densities
  - Infrared: (940 nm) for high cell densities

## Options



### CAUTION

The light emitted by the LEDs on the sensor plate is highly sensitive and can damage the iris or retina. The CGQ BioR sensor plate contains an infrared LED that emits high energy radiation in the invisible range. Sensor plates with this LED carry the warning symbol shown on the left.

- Wear safety goggles and avoid direct contact of LEDs with eyes or skin.
- Always keep a safety distance of >1 m from active sensor plates.
- Pause or stop running measurements before all work within the safety distance.

- Manufacturer: aquila biolabs
- Measures within a range of 0 to 1000

### INFORMATION

CGQ BioR sensors are optimised for microbial bioprocesses. The sensors may be used in temperatures from 15 to 50 °C.

The CGQ BioR sensors non-invasively measure the scattered light of the culture. This is proportional to the biomass concentration in the bioreactor, but can also be processed, e.g. by a soft sensor in eve®, in order to obtain a correlation with the optical density.

Details and specifications of the sensors and their measuring principles as well as safety, use and maintenance can be found in the separate documentation of the manufacturers. Read these before using the turbidity sensor and follow the instructions.

### 4.1.1 Calibrating the Sensor

#### Variant Optek

Optek sensors are pre-calibrated ex-factory. Inserts are available for reference measurement.

Due to the different light absorption of different media, zero point calibration should be performed before each cultivation process. This can be done on the operating panel, either **before or after** autoclaving, depending on the application in question. For more details, see the main chapter "Operation" chapter "Calibrating the Turbidity Sensor".

## 4.1.2 Mounting the Sensor

### Variant aquila biolabs

CGQ BioR sensors are pre-calibrated ex-factory. A new calibration is not necessary.

### Variant Optek

For culture vessels DN 90 and DN 145, Optek ASD sensors can be mounted directly into 12 mm/Pg13.5 ports. For culture vessels with DN 115, an electrode holder is used. For more details on the electrode holder, refer to main chapter "Accessories" chapter "Electrode Holder".

Note the following points for mounting:

- Ensure that the sensor is fitted with an O-ring; fit an O-ring if necessary.
- Mount the sensor by hand – do not use any tools!
- If the mounting depth of the sensor is adjustable (mounting with electrode holder), make sure the mounting depth is set correctly prior to autoclaving, as later adjustment represents a contamination risk.
- Mount the sensor in such a way that it cannot come in contact with other components or the glass vessel.
- Mount the sensor in such a way that it has good access to the flow and there is no risk of bubbles collecting in the measurement gap.

### Variant aquila biolabs

CGQ BioR sensors are always attached to the culture vessel with the strap attached to the sensor. For this purpose, the sensor with the measuring window is pressed against the glass vessel and fixed with the strap. Depending on the culture vessel, different positions of the sensor or attaching methods may be necessary. For mounting details, see separate documentation of the sensor manufacturer.

Note the following points for mounting:

- Ensure that the sensor is not attached to markings or stickers on the glass vessel, this may affect the measurement.
- Mount the sensor so that it is not in front of or in the direct vicinity of reflective steel parts (< 20 mm).

## Options

- Ensure that the sensor is positioned in such a way that liquid is in front of the measurement window during the entire bioprocess
- Foam, high gas hold-ups and the use of antifoam agents can (significantly) interfere with the light scattering of growing cells.

### 4.1.3 Interferences Turbidity Measurement

Interference		
Displayed measured value is not plausible / unusual		
Possible Cause	Remedy	By
Sensor cable is twisted or kinked or not properly connected.	Check and ensure that the sensor cable is not kinked or twisted. Connect the sensor cable properly as necessary.	Operator
<b>Optek</b> Sensor is not calibrated	Calibrate the zero point	Operator
<b>Optek</b> Window fouling on the sapphire windows.	Carefully clean the sensor	Operator
<b>aquila biolabs</b> Sensor is mounted in the wrong place / measures in foam	Place the sensor at the level of the liquid. Make sure that there are no obstacles in front of the measuring window.	Operator
Faulty sensor cable	Replace sensor cable	Infors service technician
Faulty sensor	Replace the sensor	Operator

## 4.2 Exit Gas Analysis

In order to allow the user to draw conclusions regarding the status of the culture while the bioprocess is still underway, the CO<sub>2</sub> and O<sub>2</sub> measurements are often taken and analysed in the exit gas flow of the bioreactor.

### 4.2.1 Gas Sensor

Combined CO<sub>2</sub> and O<sub>2</sub> sensors of the type BlueInOne Ferm, BlueInOne Cell or BlueVary are available for exit gas analysis.

**Measurement range gas sensors**

Sensor type	Vol. % O <sub>2</sub>	Vol. % CO <sub>2</sub>
BlueInOne Ferm BlueVary	1.0 – 50 <sup>1)</sup>	0 – 10
		<i>or</i>
		0 – 25
BlueInOne Cell BlueVary	0 – 100 <sup>2)</sup>	0 – 10
		<i>or</i>
		0 – 25

<sup>1)</sup> suitable for aerobic bioprocesses only

<sup>2)</sup> suitable for aerobic and anaerobic bioprocesses

3 m of pressure hose, D = 8 x 14.5 and a clamp are included for connecting the gas sensor to the culture vessel (exit gas filter).

For details on the safety, technical data, usage and maintenance requirements for the gas sensors, see the separate documentation provided by the sensor manufacturer. Read this documentation before using the gas sensor and follow the instructions contained therein.

## 4.2.2 Connecting the Gas Sensor

In order to view measurements on the operating panel, the gas sensor must be connected to the sensor cable, and the exit gas from the bioreactor must be led through the gas sensor using a hose. The cable is usually connected once during commissioning and can remain untouched thereafter. The connection to the exit gas line must be re-established before each cultivation process.

The ideal connection conditions are detailed in the separate documentation provided by the manufacturer.

### Connecting the sensor cable

The sensor cable is pre-installed on the rear of the device ex-factory. The cable has an 8-pin round plug connector. To connect the sensor, the plug connector is plugged into the socket marked **Port A** on the gas sensor.

Due to the length of the sensor cable, the gas sensor can be positioned in a large number of possible locations.

## Options

### Establishing the hose connection

The hose connection between the culture vessel (exit gas filter) and the gas sensor must be designed in line with the direction in which the gas flows through the gas sensor.

Proceed as follows:

Procedure

1. Cut as short a piece as possible off the supplied pressure hose.
2. Push one end of the hose onto the hose nozzle (observe direction of flow) on the gas sensor's flow adapter and fasten in place with the clamp.
3. Push the open end of the hose onto the exit gas filter on the exit gas cooler.



#### INFORMATION

Do NOT use a clamp here, as the hose must be easy to disconnect at this point, e.g. for autoclaving the culture vessel.

### 4.2.3 Calibrating the Gas Sensor

1-point calibration must be carried out once per month and during initial commissioning in order to guarantee exact measurement results.

This is done directly on the gas sensor itself. The procedure is described in the separate documentation provided by BlueSens.

### 4.2.4 Replacing the BlueVary Gas Sensor Cartridge

The max. operating time of a BlueVary gas sensor cartridge amounts to 9000 operating hours. Once this limit is reached, measurement is no longer possible. I.e. there is no measurement value output anymore and the display turns red. The gas sensor cartridge must be replaced by the sensor manufacturer.

## 5 Accessories

The table below lists all the accessories included in the standard package, divided according to vessel size (TV = total volume) and nominal diameter (DN), inside diameter) as well as version of the device. M = version for microorganisms, C = version for cell culture.

Accessories	1.5 L TV/ DN 90		3.0 L TV/DN 115		6.0 L TV/ DN 145	
	M	C	M	C	M	C
Impeller, Rushton	2	--	2	--	2	--
Impeller, pitched bladed, downward flow	--	1	--	1	--	1
Baffles	1	--	1	--	1	--
Sparger, ring-shaped	1	1	1	1	1	1
Immersion pocket for temperature sensor in 10 mm port	1	1	1	1	1	1
Dip tube, straight, Ø 6 mm for 12 mm/Pg13.5 port	1	1	1	1	1	1
Addition port adapter, for 7.5 mm port	4	4	4	4	4	4
Clamping adapter for 10 mm port	3	3	3	3	3	3
Antifoam sensor for 10 mm port	1	1	1	1	1	1
Blanking plug for 12 mm/Pg13.5 port	4	4	6	6	7	7
Blanking plug for 10 mm port (in starter kit)	2	2	2	2	2	2
Exit gas cooler for 12 mm/Pg13.5 port	1	1	1	1	1	1
Reagent bottle, 250 mL	4	4	4	4	4	4
Pump heads with hoses Inside diameter: 1.0 mm/wall thickness: 1.1 mm	4	4	4	4	4	4
pO <sub>2</sub> sensor (sensor type according to existing measurement system)	1	1	1	1	1	1
pH sensor (sensor type according to existing measurement system)	1	1	1	1	1	1
Electrode holder for 12 mm/Pg13.5 port (in starter kit)	--	--	2	2	--	--
Starter kit	1	1	1	1	1	1
Cone plug for drive hub (in starter kit)	1	1	1	1	1	1

## Accessories

### 5.1 Cone Plug for Drive Hub



The cone plug (EPDM) provided in the starter set protects the drive hub from penetration of condensate water during sterilisation in the autoclave.



It must be plugged into the opening of the drive hub for autoclaving of the culture vessel!

### 5.2 Sparger



The gas is fed directly into the medium via a ring sparger ( $\varnothing$  6 mm) with evenly distributed holes on the bottom side of the ring through which the air/gas bubbles into the culture medium.

Inside diameter	4.0 mm
Hose connection outside diameter	6.0 mm

### 5.3 Baffles



The baffles are used to mix the culture in culture vessels for microorganisms. They are simply inserted into the glass vessel.

### 5.4 Blanking Plugs

Blanking plugs are used to seal open ports. There are different blanking plugs for the different types of port.

#### **Blanking plug, Ø 10 mm**

Fitted with fixed O-ring.

A fastening screw is used to fasten it in the 10 mm vessel top plate port (see the "Clamping Adaptors and Fastening Screws" chapter).



## Accessories

### Blanking plug, Ø 12 mm

Must be fitted with an O-ring before being mounted in the 12 mm/Pg13.5 port.

Mounted using a thread.



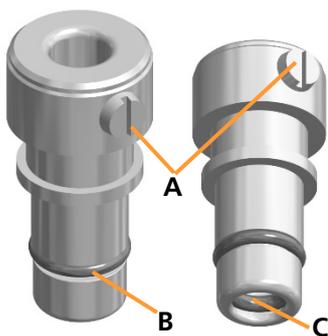
## 5.5 Clamping Adapters and Fastening Screws

Clamping adapters are used when mounting the sparger, the various dip tubes and the antifoam/level sensors. The clamping adapter fixes the component in place and can be used to adjust its mounting depth.

The clamping adapter must match the outside diameter of the part being installed and the size of the port in the vessel top plate.

### Clamping adapter Ø 6/10 mm

Fitted with two fixed O-rings (B & C).



When the slotted-head screw is undone, the component with a diameter of 6 mm can be inserted in or removed from the clamping adapter. When the slotted-head screw is tightened, the component is clamped in the clamping adapter.

**Fastening screw M5**

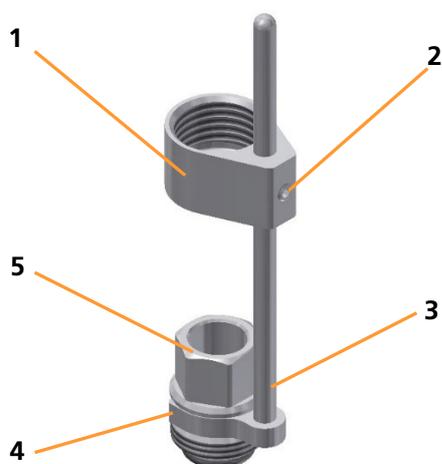
The fastening screws are used to hold components in place in the Ø 10 mm ports in the vessel top plate.



**5.6 Electrode Holders**

Electrode holders are used to adjust the mounting depth of sensors (pH, pO<sub>2</sub>, etc.) in 12 mm/Pg 13.5 ports. The electrode holder, respectively the sensor must be fitted with an O-ring for mounting.

The electrode holder comprises a sheath with a grub screw, a guide bar with a fork, and a hollow screw. The wrench for the grub screw is also included in the scope of supply.



- 1 Sheath
- 2 Grub screw
- 3 Guide bar
- 4 Fork
- 5 Hollow screw

**5.7 Addition Port Adapters and Feed Needles**

Addition port adapters and feed needles are used to feed liquid into the culture vessel or are used for head space gassing (version for cell culture), too. They each come with a hose connection, are fitted with a fixed O-ring and are mounted into the four 7.5 mm ports in the vessel top plate.

A single fastening screw is used to fasten all four addition port adapter(s) and/or feed needle(s) in place.

## Accessories



### Addition port adapter Ø 7.5 mm

Inside diameter	2 mm
Hose connection outside diameter	4 mm
Installation depth	17 mm

Addition port adapters protrude as far as the head space of the vessel, and have very sharp ends with slanted points.

Each culture vessel comes with four addition port adapters as standard.

### Feed needle Ø 7.5 mm

Inside diameter	2 mm
Hose connection outside diameter	4 mm

Feed needles protrude to below the minimum fill level (= min. working volume) of the culture vessel.

This method of adding liquid allows more precise and regular dosing even when handling small volumes as, unlike the port adapter, the feed needle does not drip.



#### INFORMATION

The illustration does not show the whole length of the feed needle.



## 5.8 Septum Collar



The septum collar with inside thread is used to inoculate the culture in combination with the syringe, injection needle and septum (inoculation membranes); see the “Inoculation Accessories” chapter. The septum collar is used to hold the septum in place in the 12 mm/Pg13.5 port.

## 5.9 Dip Tubes

Dip tubes are open at both ends and are mounted in a vessel top plate port with a clamping adapter.

Dip tubes are used for a variety of purposes:

- For filling the culture vessel after autoclaving. Using a dip tube prevents foaming.
- For adding inoculum.
- For sampling. The aseptic Super Safe Sampler system can be used for sampling.
- For harvesting
- For siphoning off medium during continuous cultivation
- For draining the culture vessel

Depending on the purpose, silicone hoses are connected to the dip tube via other vessels, sampling systems or, if necessary, hose networks.

Multiple dip tubes can be used at any one time, providing that enough vessel top plate ports are available.

## Accessories



### Dip tube, straight, Ø 6 mm

Inside diameter	3.0 mm
Hose connection outside diameter	4.0 mm

The dip tube does not reach as far as the bottom of the vessel.



#### INFORMATION

The illustration on the left shows only the upper section of the dip tube.

## 5.10 Immersion Pocket for Temperature Sensor (Pt100)

The immersion pocket is a tube with a sealed bottom end, and is used to insert the temperature sensor.

### Immersion pocket Ø 10 mm

Fitted with fixed O-ring.

A fastening screw is used to fasten it in the 10 mm vessel top plate port.



#### INFORMATION

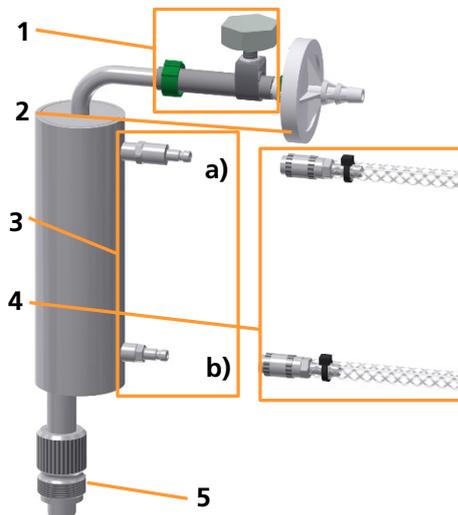
The illustration on the left does not show the full length of the immersion pocket.

## 5.11 Exit Gas Cooler

The basic unit supplies cooling liquid to the exit gas cooler. The cooling liquid flow rate can be adjusted using the control valve on the basic unit. The two hoses for water supply (bottom) and return (top) are connected to the basic unit at the factory and simply connected to the exit gas cooler via the two rapid couplings. The different hose lengths prevent wrong connection.

A piece of pressure hose is fitted onto the exit gas cooling pipe and equipped with an exit gas filter. The hose connections and the exit

gas filter are secured with hose clamps. The hose clamp securing the exit gas filter is equipped with a screw that can be used to loosen or tight it manually.



- 1 Pressure hose und hose clamp
- 2 Exit gas filter
- 3 Hose connections:
  - a) Water outlet
  - b) Water inlet
- 4 Hoses with rapid couplings for water supply and return (connected before delivery!)
- 5 Screw thread

The exit gas cooler is fitted with an O-ring before installation. A screw thread is used to mount it in the 12 mm/Pg13.5 vessel top plate port.



**INFORMATION**

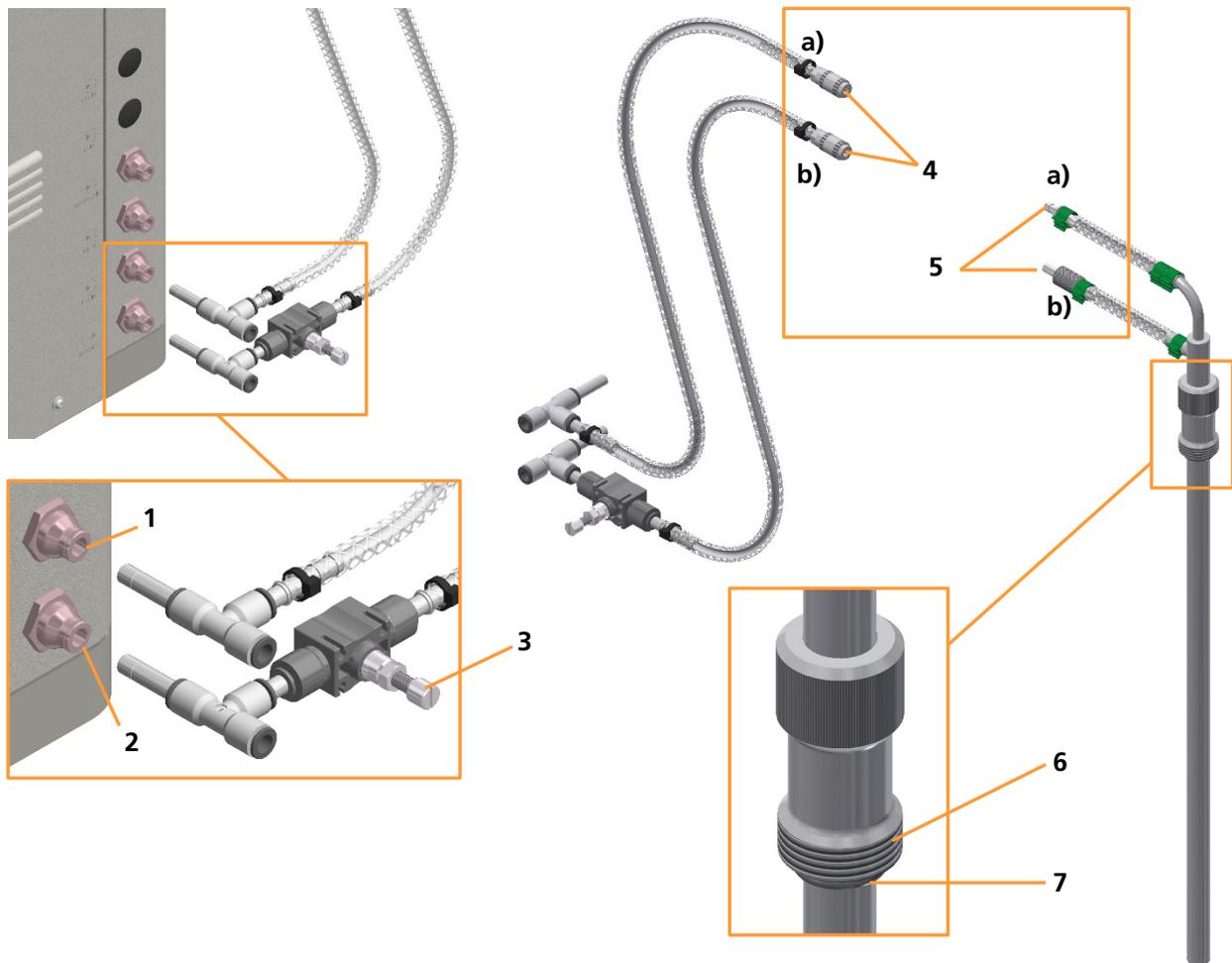
The exit gas filter must be replaced with a new filter after each cultivation process.

The exit gas cooler only works when the temperature control system is switched on.

**5.12 Cold Finger**

For microbial bioprocesses with very high waste heat, a cold finger can be used to increase the cooling capacity. The cold finger is connected directly to the water supply of the device. The flow rate is adjusted manually via a valve.

## Accessories

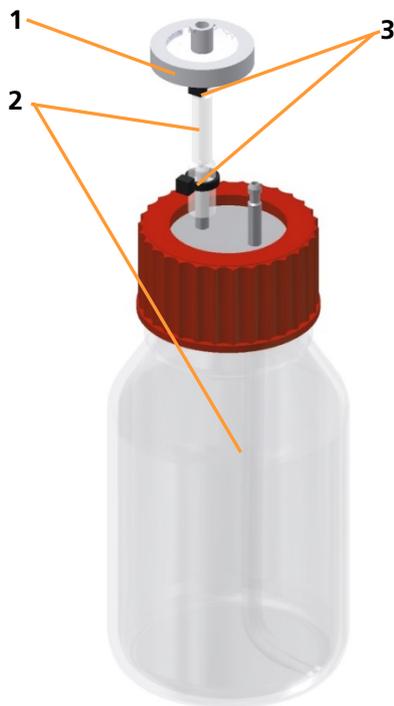


- |   |                                                          |   |                                                   |
|---|----------------------------------------------------------|---|---------------------------------------------------|
| 1 | Connection <b>H2O IN</b> : water inlet                   | 5 | Plug nipple, water inlet (a) and water return (b) |
| 2 | Connection <b>H2O OUT</b> : water outlet                 | 6 | Screw thread                                      |
| 3 | Manual control valve for water flow                      | 7 | O-ring                                            |
| 4 | Rapid coupling DN6, water inlet (a) and water return (b) |   |                                                   |

The cold finger is supplied ready for use. For mounting in the 12 mm /Pg13.5 port a screw thread is used.

The two hoses for water inlet and return are two-part. They are connected to each other for operation via rapid couplings and separated there for autoclaving the culture vessel. The T-connectors at the hose ends also serve as coupling pieces for the connection of the pressure hoses for water supply and return of the device.

### 5.13 Reagent Bottles



Two sizes of borosilicate reagent bottle are available for adding reagent and feed solution:

- 250 mL
- 500 mL

Reagent bottles are fitted before delivery. There are two hose connections on the lid. One is fitted with a short piece of silicone hose with a filter for pressure equalisation.

The second connection is fitted with a piece of silicone hose at the other end, inside the bottle.

A 2 m piece of hose is included in the scope of supply for connecting the reagent bottle to the addition port adapter in the culture vessel and to a pump head.

- 1 Filter
- 2 Silicone hose  $\varnothing = 2 \times 6$  mm
- 3 Cable tie



#### INFORMATION

250 mL reagent bottles are supplied as standard in the unit package. These fit in the reagent bottle holder that is built into the vessel holder.

The reagent bottles are equipped with filters and hoses of the correct length and connected to the pump heads ex-factory.

### 5.14 Sampling System Super Safe Sampler

Basically different systems and also individual components are available for sampling. This operating manual describes the operation and handling of the aseptic sampling system Super Safe Sampler combined with a dip tube.

The use of the Super Safe Sampler prevents the culture vessel from contamination when sampling.

## Accessories

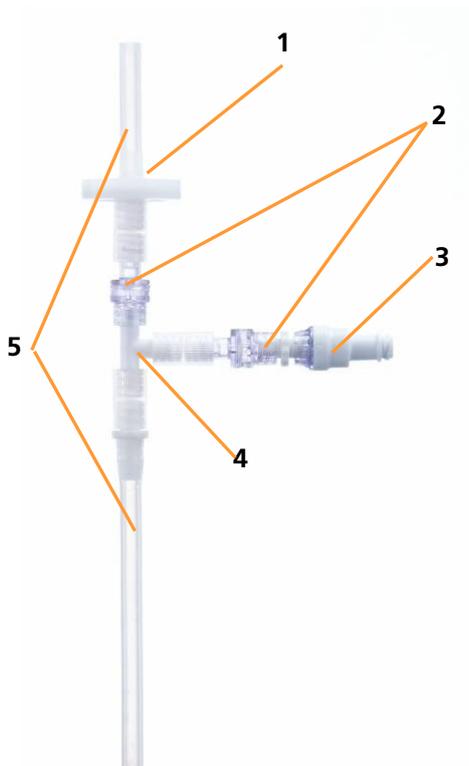
### Content of the set

The set consists of a completely pre-assembled group of valves with hoses and two syringes. It is connected via silicone hose with a dip tube.

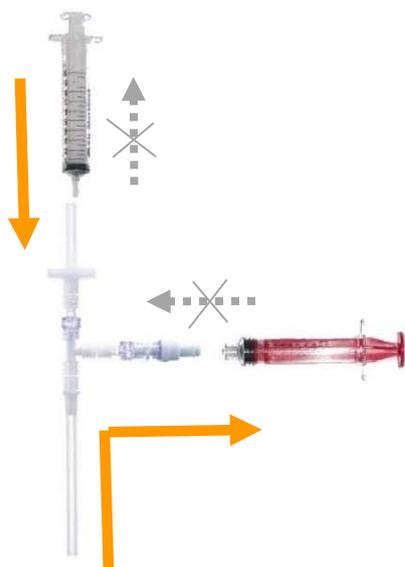


### Valve assembly

- 1 Sterile filter
- 2 Check valve
- 3 Luer activated sample valve
- 4 T-piece
- 5 Hose



The valve assembly consists of a T-piece, two check valves, a Luer-activated automatic sample valve, a sterile filter, a length of hose as an adapter for the syringe and another hose for connection to the sample dip tube in the culture vessel.



### Principle of function

The sample valve on the side arm of the T-piece opens by putting the Luer connector of the syringe into the valve and closes by removing the syringe. No further handling is necessary.

Unintentional re-introduction of the sample material once it has been withdrawn is prevented by a check valve. Thus, contamination of the bulk culture is impossible.

Following sampling, a second syringe can be fitted and air pushed in via the sterile filter, in order to displace culture solution from the sample hose and the dip tube of the vessel. With a conventional sampling system, the next sample cannot be taken immediately, as rinsing of the sampling hose and the immersion tube is necessary. By previously removing most of the culture in the sampling line, this sampling system can save culture volume, which is particularly important with small vessels and/or frequent sampling.

The dead volume of the culture remaining in the group of valves after flushing with sterile air amounts to a few  $\mu\text{l}$  and is negligibly small. If the withdrawal of a very small sample volume is required, with minimum possibility of falsification, a small quantity of culture solution (e.g. 1 ml) can be introduced and rejected before the actual sample is taken.

### Designated use

The Super Safe Sampler is designed for aseptic sampling of completely liquid samples.

Solid parts in the sample may lead to clogging of the valves. Therefore, employing the Super Safe Sampler for solid media is not recommended.

The Super Safe Sampler is autoclavable (not the syringes!) and for this reason reusable.

### Practical tips for the use of the Super Safe Sampler

Sterility of the culture vessel is ensured at all times without the possible measures mentioned below.

## Accessories

The use of a sterile syringe and sterile caps is only necessary if the sample has to be processed under sterile conditions. For sampling, the same non-sterile syringe can be used repeatedly, without fear of contamination of the culture vessel.

### Aseptic Sampling

For each sample, use a new, sterile syringe with Luer Lock fitting, in order to ensure the sterility of the sample.

Sterile syringes are consumables and therefore not included in the set.



#### INFORMATION

The use of another syringe is also possible. But a syringe with Luer lock prevents unwanted movement of the syringe.

- Before fitting the syringe, disinfect the sample valve. For this, spray a commercially available disinfectant onto the valve.
- After spraying and after each sampling, close the the sample valve with a sterile Luer-Lock cap (Dead End Cap) to keep the valve and sample sterile.

The caps are not included in the kit. Very convenient to use are so-called combi-caps that fit on male and female connectors alike.

Caps that are vented and made of steam sterilisable material can also be fitted during autoclaving.

## 5.15 Pump Heads

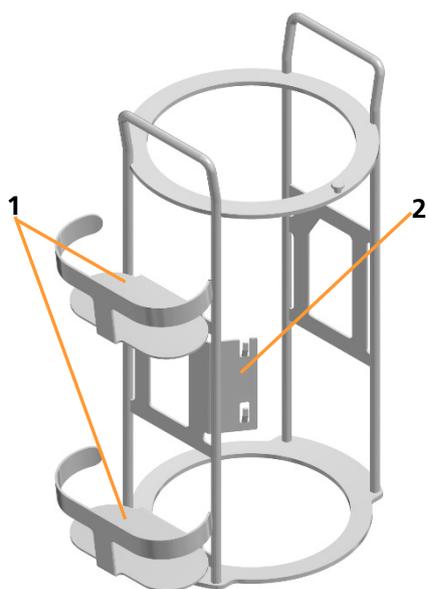


The autoclavable pump heads are fitted with PharMed pump hoses prior to delivery. Three different hose diameters are available for different delivery rates:

- 1.0 mm (standard)
- 0.5 mm
- 2.5 mm

For more detailed information about pumps and hoses refer to main chapter "Technical Data", chapter "Specifications", "Pumps".

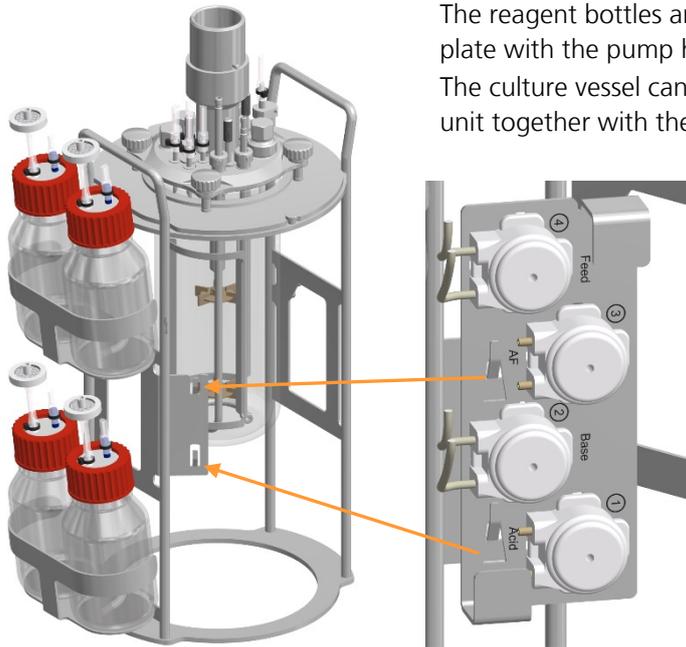
## 5.16 Vessel Holder with Built-in Holder for Reagent Bottles and Pumps



The vessel holder frame has a holding device for four reagent bottles and a holder for the four pump heads.

- 1 Reagent bottle holder
- 2 Pump holder

## Accessories



The reagent bottles are placed in the two holders, and the mounting plate with the pump heads is simply pushed onto the pump holder. The culture vessel can thus be transported and autoclaved as a single unit together with the reagent bottles and pump heads.

### 5.17 Sterile Filters

Sterile filters are used to protect against contamination in both the gassing line and the exit gas line. In addition to this, all reagent bottles used for pressure equalisation must be fitted with a short piece of hose with a filter.

All the sterile filters in the scope of supply are autoclavable, disposable filters with PTFE diaphragms.

#### **i** INFORMATION

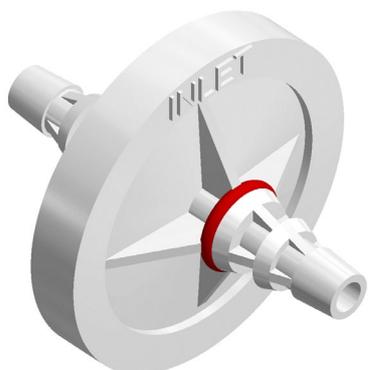
Sterile filters must be clean and dry at all times, and should thus ideally be replaced after each use.

#### **Ø 37 mm, marked red**



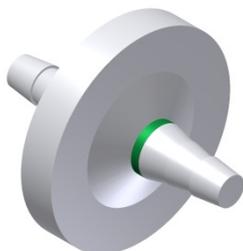
Application	<ul style="list-style-type: none"> <li>■ Version for cell culture: sparger &amp; head space gassing all vessel size</li> <li>■ Version for microorganisms: sparger gassing 1.5 L culture vessels</li> </ul>
Retention rate	0,2 µm

**Ø 50 mm, marked red**



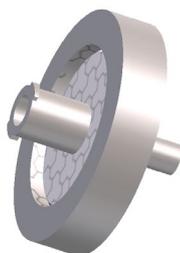
Application	Version for microorganisms: sparger gassing, 3.0 & 6.0 L culture vessels
Retention rate	0,2 µm

**Ø 37 mm, marked green**



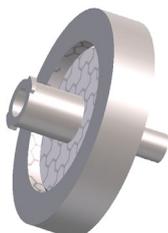
Application	Exit gas
Retention rate	0.3 µm dry 1.0 µm wet

**Ø 25 mm, not marked**



Application	Super Safe Sampler
Retention rate	0.2 µm

**Ø 25 mm, not marked**



Application	Reagent bottles (pressure equalisation)
Retention rate	0.45 µm
Diaphragm	PTFE

## Accessories

### 5.18 Hoses and Accessories

The following hoses and accessories, such as hose clamps and brackets, are available:

Hose type	Ø mm	Application
Pressure hose, fibre-glass-woven	6 x 11.9	Water and gas connections (on-site)
Pressure hose, fibre-glass-woven	6 x 10	Exit gas filter attachment (on exit gas cooler)
Pressure hose, transparent	5 x 10	Inlet air filter attachment on sparger for 3.0 and 6.0 L culture vessels for microorganisms
Silicone hose	5 x 8	Hose line from basic unit to inlet air filters for all culture vessels. Inlet air filter attachment on sparger <sup>1)</sup>
Silicone hose	3 x 6	Inlet air filter attachment for head space gassing (version for cell culture)
Pressure hose, transparent	4 x 8	Water supply and return, exit gas cooler
Silicone hose, transparent	2 x 6	Reagent bottles

<sup>1)</sup> *Version for microorganisms: 1.5 L culture vessel, version for cell culture: all vessel sizes.*

Attachments	Application
Hose clamp, screw with screwdriver slot, 14 mm, INOX	Water and gas connections (on-site) Exit gas filter attachment (on exit gas cooler)
Hoffmann pinchcock, 12 mm, nickel-plated brass	To clamp off hose lines, e.g. unused addition port adaptors/feed needles, sparger hose line, etc.
Cable tie, 2.4 x 85, polyamide	Hoses for reagent bottles and pumps, inlet air filter, sparger, water supply and return for exit gas cooler, sampling system dip tube
Hose connector, 3/32 " x 1/16 ", PVDF	Pump heads with hoses to reagent bottles

## 5.19 O-Rings and Gaskets

Designation	Ø mm	Application
O-ring, EPDM	3.53 x 94.84	Top plate gasket, culture vessel, DN 90
O-ring, EPDM	3.53 x 120.24	Top plate gasket, culture vessel, DN 115
O-ring, EPDM	3.53 x 148.8	Top plate gasket, culture vessel, DN145
O-ring, EPDM	2.62 x 10.77	Gasket, port size 12 mm/Pg13.5
O-ring, EPDM	1.5 x 7.5	Gasket, port size 10 mm
O-ring, EPDM	1.5 x 5.0	Gasket, port size 7 mm
O-ring, EPDM	1.78 x 5.28	Inner gasket for clamping adaptor for 10 mm ports
PTFE ring	120 x 105	Damping ring between glass vessel and vessel holder, DN 90
PTFE ring	145 x 130	Damping ring between glass vessel and vessel holder, DN 115
PTFE ring	175 x 160	Damping ring between glass vessel and vessel holder, DN 145
Flat gasket, silicone	32 x 42 x 2	Gasket for reagent bottle lid (all sizes)

## 5.20 Inoculation Accessories and Tools

The following inoculation accessories and tools are used:

### Accessories for inoculation

Septum, Ø = 16 mm MVQ silicone, transparent, for 12 mm/Pg13.5 ports

Sterile disposable syringe, Luer, 10 mL, inside Ø 14.35 mm

Sterile hollow needle, 20G, L = 40 mm/Ø = 0.9 mm

## Accessories

### Tools for screws, grub screws and blanking plugs

Hex key, WAF 2, DIN911

Grub screws impellers 3.0 and 6.0 L culture vessels

Hex key, WAF 1.27

Grub screws impellers 1.5 L culture vessels

Hexagon socket spanner, WAF 17

Blanking plugs 12 mm/Pg13.5 ports

Torx screwdriver, TX25

Screws thermal block adapter

### 5.21 Starter Kit

Each device package comes with a starter kit with a variety of hoses, attachments, inoculation accessories and tools. A detailed contents list is included in each starter kit.

### 5.22 Service Sets

Service sets with O-rings, gaskets, sterile filters, hoses, etc. to fit each vessel size are available separately. A detailed contents list is included in the service set.

### 5.23 Auxiliary Supplies

The term "auxiliary supplies" covers all the substances and materials required for operation and/or maintenance that cannot be considered part of the device or the system.

#### pH Buffers

pH buffers are used to calibrate the pH sensors. 250 mL bags are available for the following buffers:

- pH 4.04
- pH 7.01

## 6 Transport and Storage

The following specifications are based on transport and storage of an unpacked device at the provider's site.

### 6.1 Transport



#### WARNING

Improper transport, the use of incorrect auxiliary equipment and careless handling of the device may lead to injuries and severe property damage.

The following points must be observed when transporting the device internally (relocation):

- Always work in pairs and use suitable auxiliary equipment when transporting the device.
- The entire device (basic unit and culture vessel) contains delicate glass parts.
- Especially when using auxiliary tools, it is important to observe that the device's centre of gravity is not in the middle.



#### WARNING

The entire device (basic unit and culture vessel) is too heavy to be carried by one person alone.

Even the basic unit on its own exceeds the weight that should be carried by one person alone.

## Transport and Storage

### 6.2 Storage

- Before each time they are put into storage, decontaminate, thoroughly clean and dry the culture vessel and all accessories <sup>1)</sup>.
- Store the device and its components clean, dry and protected against dust, dirt and liquids.
- Store the device and its components in a cool place with low air humidity but protected against frost.
  - Storage temperature: 5 °C to 55 °C
  - Relative air humidity, non-condensing: 10 % to 95 %.
- Protect the device from aggressive media, direct sunlight and mechanical vibrations.

<sup>1)</sup> *Maintain and store sensors produced by other manufacturers in accordance with the separate documentation.*

## 7 Installation and Initial Operation

To set up and connect the device, note the following:



### WARNING

Faulty installation may lead to dangerous situations or severe loss of property.

Follow the installation and commissioning instructions in this operating manual precisely.

### 7.1 General Location Requirements for Installation

The following requirements must be met for the installation of the device:

- The figures and ranges specified in main chapter “Technical Data”, chapters “Connection Values” and “Operating Conditions” must be observed.
- The device must only be installed inside a laboratory or a laboratory-like environment.
- The installation site must be level, sufficiently stable and able to bear loads.
- There must not be any sources of electrical interference near the device.

### 7.2 Minimum Distances

To operate and maintain the device it must be installed with a minimum spacing of 150 mm from walls, ceilings or other equipment.

### 7.3 Connecting the Device to On-Site Supply Lines

The following chapters describe which connection requirements must be fulfilled on site and how the device is connected to on-site supply lines.

## Installation and Initial Operation

### 7.3.1 Power Supply

#### Connection conditions

The in-house power supply to the device must meet the following requirements:

- Single-phase, constant power supply
- 120/230 VAC 50/60 Hz

#### Connection

To connect the device to the in-house power supply, proceed as follows:

Procedure

1. Insert the power cable supplied into the connector socket on the device.
2. Insert the cable into the in-house power supply.

### 7.3.2 Water Supply and Return

#### Connection conditions

The in-house water supply to the device, as well as the drainage of the water, must meet the following requirements:

- "Very soft" or "soft" water quality ( $\text{CaCO}_3$  concentration 0 mmol L<sup>-1</sup> to 1.5 mmol L<sup>-1</sup>)



#### ATTENTION

Not observing the water quality requirements may lead to damage or failure of the device.

- Constant water supply at a pressure of  $2 \pm 1$  bar
- Manometer to check the primary pressure available
- The drain is heat-resistant and without back pressure

#### Connection

To connect the device to the in-house water supply and drainage, proceed as follows:

Procedure

1. Cut the required quantity of the supplied pressure hose ( $\varnothing = 6 \times 11.9$  mm).

## Installation and Initial Operation

2. Position the pressure hoses on the appropriately marked hose nozzles on the device.
3. Connect the hoses to the in-house water supply and drainage.
4. Secure the hoses with hose clamps to prevent slipping.
5. Check to ensure that the hoses neither have kinks nor are able to kink and that connections and hoses do not have any leaks.

### 7.3.3 Gas Supply

#### Connection conditions

The in-house gas supply to the device must meet the following requirements:

- Constant gas supply at a pressure of  $2 \pm 0.5$  bar
- Gas(es) is/are dry, clean and free of oil and dust
- Recommended compressed-air quality as per DIN ISO 8573-1: Class 1,2,3,4



#### ATTENTION

The use of impure gases can lead to blockage of the sterile filter and damage the mass flow controller.

Only use dry, clean and oil-free gases.

#### Connection

To connect the device to the in-house gas supply, proceed as follows:

#### Procedure

1. Cut the required quantity of the supplied pressure hose ( $\varnothing = 6 \times 11.9$  mm).  
Only use hoses supplied by the manufacturer.
2. Position the pressure hoses on the appropriately marked hose nozzles on the basic unit.
3. Connect the hoses to the in-house gas supply.
4. Secure hoses with hose clamps to prevent slipping.
5. Check to ensure that hoses neither have kinks nor are able to kink and that connections and hoses do not have any leaks.

## Installation and Initial Operation



### WARNING

The use of inappropriate or damaged hoses and/or inappropriate fixing may lead to leakage of gases. Depending on the gas in question, there may be a danger of gas explosion and/or danger of suffocation as well as a hazard for the health of the operator.

Always close the gas supply before a hose is removed and when the device is not in use.

### 7.3.4 Exit Gas

Ensure the following on the house side:

- The exit gas is safely discharged by means of a suitable, gas-tight hose.
- The working environment is equipped with an adequate ventilation system, depending on the application.

## 7.4 Connecting the Motor Cable

The motor is controlled directly by the basic unit and is connected to it via the motor cable.

For routine operation, it is not necessary to plug in and unplug the motor cable. The connected motor is only coupled before cultivation. For details, see the main chapter "Before Cultivation", chapter "Connecting the Motor".

To connect the motor cable, proceed as follows:

Procedure

1. Ensure the device is switched off.



### ATTENTION

If the motor cable is connected to or disconnected from the motor while the device is switched on, there is a risk of a short circuit that could damage the control electronics.

2. Insert the (angled) plug of the motor cable into the socket on the rear of the basic unit and tighten the coupling nut by hand.
3. Insert the other plug into the socket on the motor tighten the coupling nut by hand.

## Installation and Initial Operation



### Version for microorganisms

Depending on the size of the culture vessel, the large (left-hand) or small (right-hand) motor is supplied.



### Version for cell culture

The same motor is used for all vessel sizes.

## 7.5 Test Run

In order to become familiar with the basic functions of the device before the first cultivation, a short test run can be executed. The test run comprises:

- Temperature control (cooling / heating)
- Stirring
- Gassing

Compressed air of the stated quality (see chapter "Gas Supply") is used for gassing.

To avoid calcium deposits, demineralised water is recommended for filling the vessel.

The following description of the test run does not detail handling of individual components. Detailed descriptions of their handling are given in the corresponding chapters of the main chapter "Before Cultivation".

For details on operation, see the main chapter "Operation".

## Installation and Initial Operation

### 7.5.1 Preparation Test Run

Before starting the test run, check and ensure the following:

- The device is correctly connected to the water, power and gas supply and is operational
- The motor cable is connected to the basic unit and the motor.

The following work is to be executed before the test run:

Procedure

1. Remove the vessel top plate and put it aside carefully.



#### ATTENTION

If the vessel top plate presses against long components such as the stirrer shaft etc., they could bend because of the weight of the top plate.

Always position the vessel top plate so that it does not lie on top of components.

2. Fill the culture vessel with water – preferably demineralised – to the working level.
3. Ensure that the stirrer and sparger, and one addition port adapter, if applicable, are mounted; if necessary, mount them.
4. Fit the top plate and secure it.
5. Screw the exit gas cooler into the port on the vessel top plate port.

The exit gas cooler is equipped with a new exit gas filter in the factory.

6. Connect the exit gas cooler to pre-fitted hoses on the basic unit; to this end, follow the symbols on the basic unit:  
water inlet on bottom of exit gas cooler / water outlet at top of exit gas cooler.
7. Close all remaining open ports with blanking plugs.
8. Hang the culture vessel on the basic unit.
9. Connect the gassing (compressed air) to the sparger and, if applicable, to the addition port adapter, by connecting the gassing hose(s) on the basic to the nozzle(s) on the inlet air filter(s).

The sparger is equipped with a hose and the inlet air filter ex-factory. The version for cell cultures additionally has an addition port adapter equipped with a hose and the inlet air filter for head space gassing.

## Installation and Initial Operation

The gassing hose(s) is/are attached to the basic unit ex-factory.

10. Insert the temperature sensor as far as it will go into the pocket in the top plate.
11. Couple the motor.
12. Switch on the device at the power switch and wait until the system has started up.

### 7.5.2 Cooling System

To activate the cooling system, proceed as follows:

Procedure

1. On the operating panel, set a low setpoint for the *Temperature* parameter, e.g. 10 °C, in order to activate the water supply to the temperature control system.
2. Start the Batch (process) using **Start Batch** and switch on the *Temperature* parameter.
3. All parameters except for *Temperature* remain switched off; switch them off if necessary.

You should now hear water flowing into the temperature control system.

The water supply to the exit gas cooler should be activated, too now.

4. Use your hands to check whether the exit gas cooler and thermal block and/or adapter are beginning to cool down.

As soon as the temperature control circuit is full, water will flow out of the water outlet (*H2O OUT*) of the basic unit.

For the rest of the procedure, allow the Batch to run with the temperature control switched on.

### 7.5.3 Stirring

Batch is running with temperature control switched on.

To test the stirrer, proceed as follows:

Procedure

1. On the operating panel for the *Stirrer* parameter, set a low setpoint, e.g. 200 min<sup>-1</sup>.
2. Switch on the *Stirrer* parameter.

For the rest of the procedure, allow the Batch to run with the temperature control switched on and the stirrer running.

## Installation and Initial Operation

### 7.5.4 Heating and Adjusting Temperature

Batch is running with temperature control switched on and stirrer running.

To test the heating and adjust the temperature, proceed as follows:

Procedure

1. On the operating panel, set a high setpoint for the *Temperature* parameter, e.g. 45 °C.

The water supply for cooling is stopped; the system heats up.



#### CAUTION

Risk of minor burns if the heated thermal block and thermal block adapter are touched!

2. Wait until the temperature has adjusted to the setpoint.

For the rest of the procedure, allow the Batch to run with the temperature control switched on and the stirrer running.

### 7.5.5 Gassing

Batch is running with temperature control switched on and stirrer running.

#### Sparger gassing

To test the gassing via sparger, proceed as follows:

Procedure

1. On the operating panel for the *Total Flow* parameter, set a low setpoint, e.g.
  - Version for microorganisms: 1,0 L min<sup>-1</sup>
  - Version for cell culture: 100 mL min<sup>-1</sup>
2. Select *OnlyAir* setting for parameter *GasMix*, so that its setpoint is preset to 21 %.

If the gassing is working, air bubbles now form in the water in the culture vessel.

## Installation and Initial Operation

### Head space gassing (Version for cell culture)

To test the gassing via head space, proceed as follows:

Procedure

1. On the operating panel for the *Air Headspace* parameter, set a setpoint, e.g. 1000 mL min<sup>-1</sup>.
2. Remove the gassing hose from inlet air filter on the addition port adapter in the vessel top plate and hold the hose end on e.g. the back of the hand or on a finger, to feel the air flow.



#### INFORMATION

If the hose line is blocked for too long, the overpressure sensor may trigger an overpressure alarm Gas pressure high and switch off the gas supply for 10 seconds.

### 7.5.6 End of Test

After all parameter setpoints have been reached, the test can end here. The inoculation that now takes place during normal operation is not relevant to the test run.

Proceed as follows:

Procedure

1. On the operating panel, press **Inoculate** and then **Stop Batch** to stop the Batch (process).
2. Switch off the device at the power switch.
3. Shut off the supply lines.
4. Let the motor cool down (motor for device version for microorganisms).



#### CAUTION

Risk of minor burns if the motor, which heats up during operation, is touched!

5. Uncouple the motor from the vessel and place it on a clean and dry work surface.
6. Empty the culture vessel.

## Before Cultivation

# 8 Before Cultivation

The following chapters describe all the preparatory work before starting the cultivation process. This essentially comprises:

- Preparing and autoclaving the culture vessel:
  - Checking the gaskets (O-rings) on accessories and on the culture vessel
  - Mounting the accessories
  - Filling or moistening the culture vessel
  - Preparing sensors and accessories
  - Autoclaving
  
- Connecting the culture vessel and preparing for cultivation:
  - Hanging the culture vessel into place on the basic unit and connecting cables and hoses between the culture vessel and the device
  - Filling the vessel if necessary
  - Preparing sensors and accessories

## 8.1 Preparing and Autoclaving the Culture Vessel

All accessories required for later cultivation must be prepared and mounted accordingly and autoclaved together with the culture vessel.

Certain accessories are mounted ex-factory.

### 8.1.1 Checking Gaskets (O-Rings)

O-rings are used to seal all openings on the vessel and top plate. The top plate, its ports and all accessories are thus equipped with O-rings. Before every use, the O-rings must be checked that they are present, undamaged and correctly seated. Damaged O-rings must be replaced.

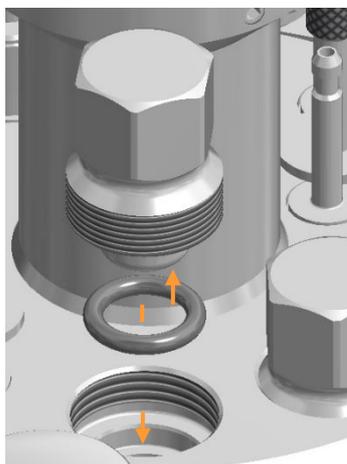


#### INFORMATION

Wet the O-rings with 70% alcohol or a little water to facilitate removing and replacing O-rings or accessories with O-rings. Do not use silicone grease; this can affect sterilisation results.

Carry out this check as follows:

Procedure



1. Check the O-ring for sealing the top plate for damage and to ensure that it is positioned correctly in the groove on the vessel flange.
  
2. Ensure that every mounting part is equipped with an intact O-ring:  
 Check that the O-rings are correctly positioned and are undamaged. If necessary, reposition or replace. If component parts are fitted into other component parts (clamping adaptor), there must also be an O-ring between them.



**INFORMATION**

Septum collars are sealed with a septum. No O-ring is used!

**8.1.2 Mounting the Impellers**

To mount the impeller(s) to the stirrer shaft, proceed as follows:

Procedure

1. Slide the impeller(s) onto the stirrer shaft.
2. Set the desired height.



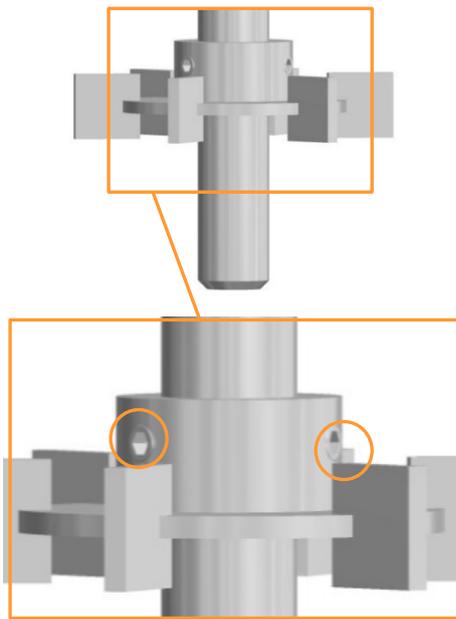
**INFORMATION**

To avoid unnecessary foam formation, do not fit the impeller at the same height as the surface of the medium.

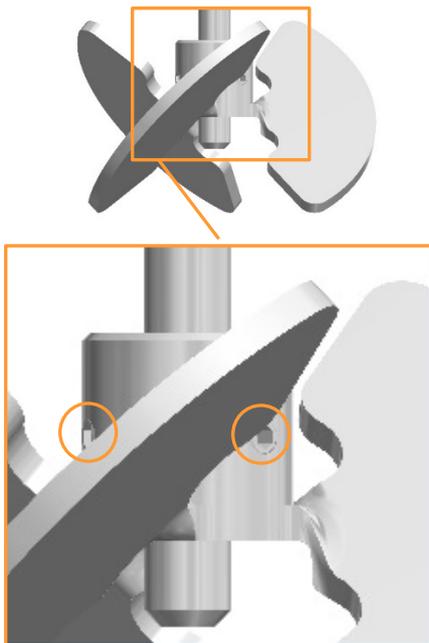
## Before Cultivation

The ideal mounting heights of both impeller types (Rushton and pitched blade) are defined ex-factory for each vessel size and can be found in main chapter „ Technical Data“, chapter “Specifications”, “Stirrer”, section “Impeller mounting heights ex-factory”.

3. Tighten the grub screws on the impeller with the Allen key.



The figure to the left shows a Rushton impeller (version for microorganisms).



The figure to the left show a pitched blade impeller (version for cell culture).

### 8.1.3 Mounting Dip Tubes and Spargers

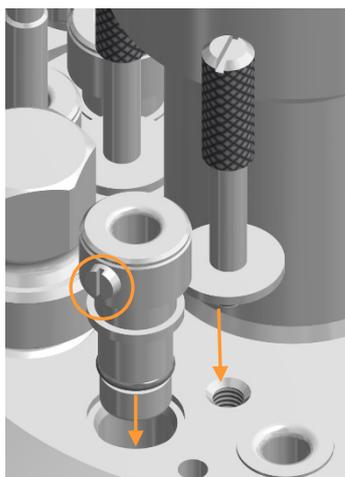
Straight spargers and dip tubes can be mounted to the outside of the vessel top plate. Curved spargers and dip tubes can only be mounted to the inside of the vessel top plate.

Since this unit uses curved spargers and straight dip tubes, mounting to the inside of the vessel top plate is described here. This means that the vessel top plate is still removed.

During mounting, ensure that the sparger or the dip tube does not come into contact with other component parts (stirrer). The sparger is positioned below the stirrer shaft.

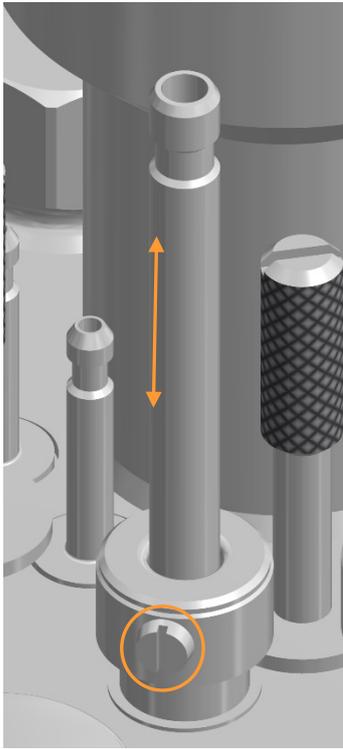
Proceed as follows:

Procedure



1. Ensure that the clamping adapter is equipped with an interior or exterior O-ring; if necessary, attach O-ring(s).
2. Insert the clamping adapter into the intended port and fix it with a fastening screw.
3. Loosen the slotted-head screw at the clamping adapter.
4. Insert the sparger/dip tube into the clamping adapter from below.

## Before Cultivation

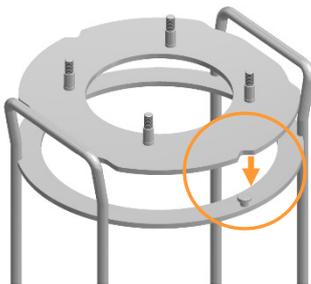


5. Set the desired mounting depth, align the sparger.
6. Tighten the slotted-head screw.

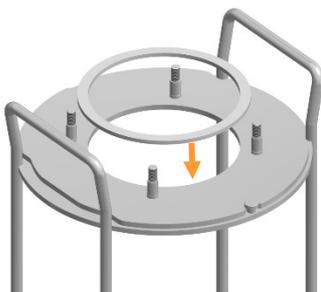
### 8.1.4 Inserting the Vessel into the Vessel Holder

To insert the glass vessel into the vessel holder, proceed as follows:

Procedure

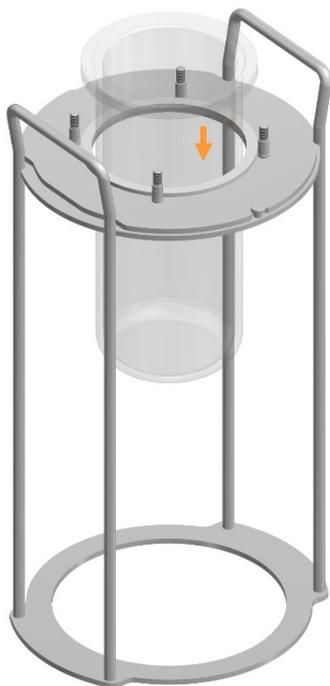


1. Place the flange onto the ring of the vessel holder:  
the two opposing recesses on the flange fit with the bolt on the ring.

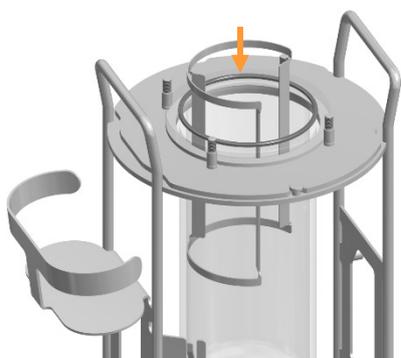


2. Place the damping ring onto the flange.

3. Insert the vessel carefully.



### 8.1.5 Inserting the Baffles



Culture vessels for microorganisms are provided with baffles. To insert them into the glass vessel, proceed with caution.

### 8.1.6 Moistening/Filling the Culture Vessel

If in the culture vessel is to be autoclaved with the medium, the vessel can be filled before the top plate is put in position and the additional component parts are mounted.

Note the following about filling the culture vessel before autoclaving:

- Before autoclaving, only top up with heat-resistant media.
- During autoclaving, evaporation may result in a loss of volume and thus to increased salt concentration in the medium. If necessary, top up with sterile water.

## Before Cultivation



### INFORMATION

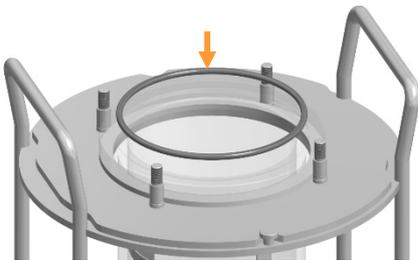
Development of steam is not possible when autoclaving an empty and dry culture vessel. Successful sterilisation is not guaranteed.

Ensure that there is liquid in the culture vessel (approx. 10 mL of water per litre of total volume).

### 8.1.7 Fitting the Vessel Top Plate

Proceed as follows to fit and fix the vessel top plate:

Procedure

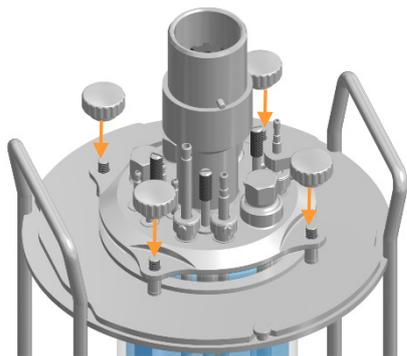


1. Fit the O-ring for the top plate gasket into the groove on the edge of the vessel.

2. Place the top plate carefully and with the correct alignment into position.

For culture vessels for microorganisms: ensure that component parts do not touch the baffles.

3. Tighten the knurled nuts on the top plate by hand (no tool!) crossways.



### ATTENTION

If the knurled nuts are tightened too much, components may be damaged, which can result in failure of the device. The knurled nuts may not be tightened with a tool under any circumstances.

This applies to all screw connections where the instructions specify that they must be tightened by hand!

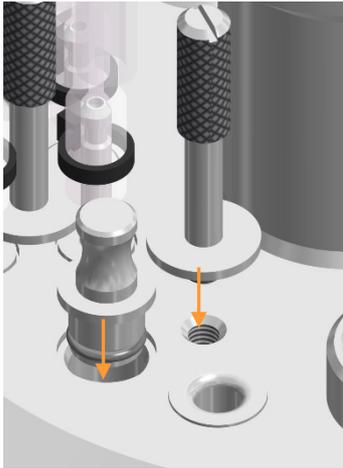
### 8.1.8 Mounting the Blanking Plugs

For mounting the different blanking plugs, proceed as follows:

#### Ø 10 mm ports

1. Insert the blanking plug with fixed O-ring into all unused ports.
2. Fix with a fastening screw.

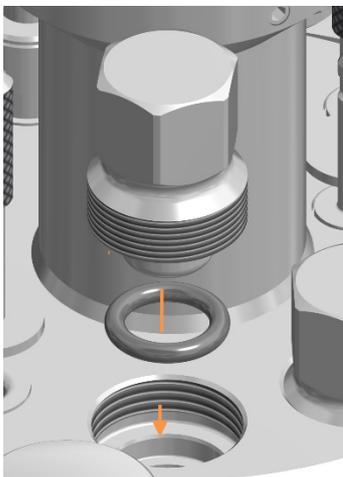
Procedure



#### Ø 12 mm / Pg13.5 ports

1. Insert the O-ring and blanking plug into all unused ports.
2. Tighten by hand.
3. Use the hexagon socket spanner to make it hand-tight.

Procedure

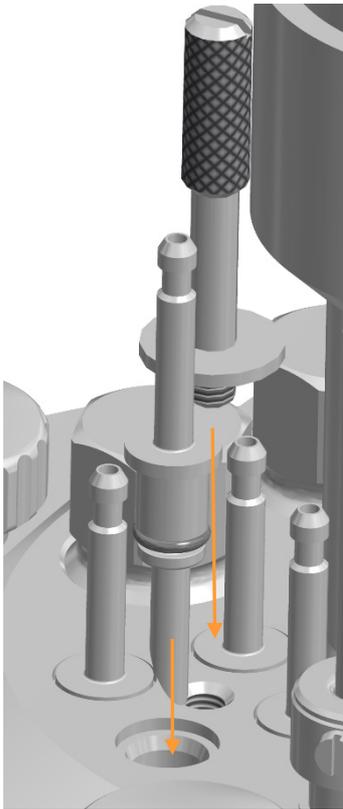


## Before Cultivation

### 8.1.9 Mounting Addition Port Adapters

Proceed as follows:

Procedure



1. Insert the addition port adapters with a fixed O-ring into the four 7.5 mm ports.
2. Fix it with the fastening screw.

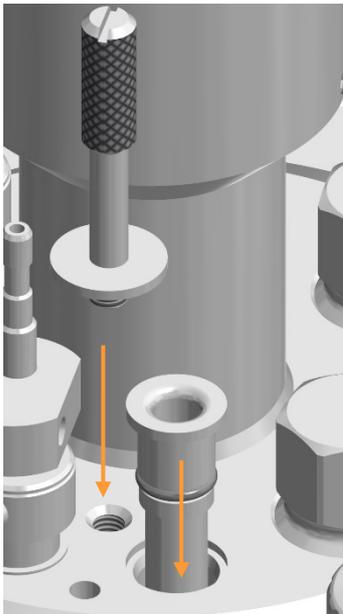
### 8.1.10 Mounting the Feed Needle(s)

The procedure for mounting one or more feed needle(s) instead of addition port adapters is the same as for the mounting of the addition port adapters. For details, see the chapter "Mounting Addition Port Adapters".

### 8.1.11 Mounting the Immersion Pocket for Temperature Sensor (Pt100)

Proceed as follows:

Procedure



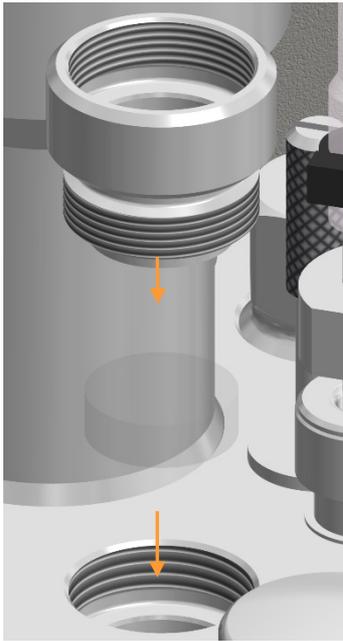
1. Insert the immersion pocket with the fixed O-ring into the 10 mm port.
2. Fix it with the fastening screw.

## Before Cultivation

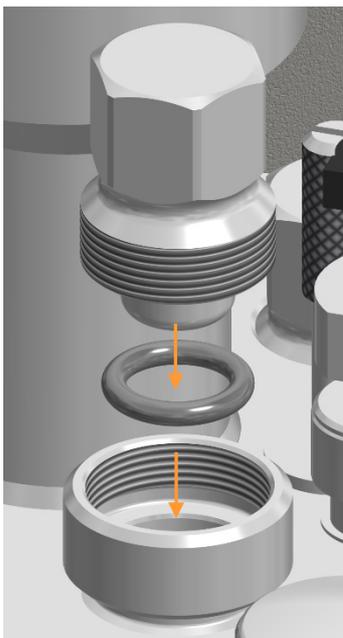
### 8.1.12 Equipping the Port with a Septum Collar and Septum for Inoculation

For later inoculation with a syringe, the 12 mm/Pg13.5 port in the top plate must be prepared as follows:

#### Procedure



1. Ensure that there is no O-ring in the port; if there is, remove it.
2. Insert the septum (inoculation diaphragm) into the port.
3. Screw the septum collar into the port by hand.



4. Insert the blanking plug equipped with an O-ring into the septum collar and screw it tight by hand.  
If necessary, use the hexagon socket spanner to make it hand-tight.

### 8.1.13 Preparing the Dip Tube/Addition Port Adapter for Inoculation

If later inoculation is to be carried out by means of a dip tube or addition port adapter, proceed as follows:

Procedure

1. Fit the dip tube with the clamping adapter or addition port adapter in the port.
2. Place a piece of silicon hose onto the dip tube/addition port adapter.
3. Equip the hose for a sterile hose connection. (Depending on the application: rapid coupling, sterile connector or weldable hose with sterile filter).
4. Secure the hose transition points with cable ties.

### 8.1.14 Mounting the Exit Gas Cooler

Mount the exit gas cooler as follows:

Procedure

1. Attach the O-ring to the thread of the exit gas cooler.
2. Screw the exit gas cooler into the 12 mm/Pg13.5 port by hand.
3. Align the exit gas cooler to ensure that handling of other component parts is impaired as little as possible.
4. Check to ensure that the exit gas filter is fitted securely.
5. Cap the exit gas filter loosely with a little aluminium foil



#### INFORMATION

A humidifier bottle with antifoam reagent can be installed between exit gas cooler and the exit gas filter if significant foam formation is expected.

Take the following into account for autoclaving:

- Only use a new, clean and dry exit gas filter and fix it in such a way that it cannot slip.
- ALWAYS keep the exit gas line - hose at the exit gas cooler with secured exit gas filter - open.

## Before Cultivation



### CAUTION

If pressure equalisation does not take place via a top plate opening or the mounted exit gas cooler, overpressure or vacuum in the culture vessel may occur during autoclaving.

### 8.1.15 Mounting the Cold Finger

When using the optional cold finger, ensure it is fitted with the O-Ring, then screw it into the 12 mm / Pg13.5 port by hand, just like the exit gas cooler. For details refer to the main chapter "Accessories", chapter "Cold Finger".

### 8.1.16 Preparing the Sensors

All sensors that come into contact with the medium are mounted before autoclaving and are sterilised together with the culture vessel.

Note the following about all sensors:

- Mount all sensors by hand – do not use any tools!
- Mount the sensors in such a way that they cannot come in contact with other components or the glass vessel.
- If the mounting depth of is adjustable (mounting with electrode holder/clamping adaptor), make sure the mounting depth is set correctly prior to autoclaving, as later adjustment represents a contamination risk.

#### pH sensor

- Calibrate the pH sensor before mounting and autoclaving.

#### pO<sub>2</sub> sensor

- Mount the pO<sub>2</sub> sensor in such a way that it has good access to the flow and there is no risk of bubbles collecting.

#### pH sensor and pO<sub>2</sub> sensor

- For vessels with a nominal width of 90 and 145: screw the sensors directly into 12 mm/Pg13.5 port.
- For vessels with a nominal width of 115: mount the sensors with an electrode holder.

**ATTENTION**

Risk of damage to the pH and pO<sub>2</sub> sensors. Covering the sensor heads with aluminium foil during autoclaving may lead to water gathering under the film, thus damage the contacts on the sensor head.

pH and pO<sub>2</sub> sensor heads may **NOT** be covered with aluminium foil during autoclaving!

For details on the safety, technical data, usage and maintenance requirements for the pH and pO<sub>2</sub> sensors, see the separate documentation provided by the sensor manufacturers.

### 8.1.16.1 Calibrating the pH Sensor

Calibration of a pH sensor must always be carried out before autoclaving.

#### Procedure

1. Connect the sensor cable. (For more details, see the chapter "Connecting the pH Sensor").
2. Switch on the device at the power switch.  
The operating panel is switched on automatically and the system is started.
3. Calibrate the pH sensor in accordance with the detailed description in the main chapter "Operation", chapter "Calibrating the pH Sensor".

**INFORMATION**

If the pH sensor has already been calibrated before connection to the system, the bioreactor will use this data and calibration using the operating panel is no longer necessary.

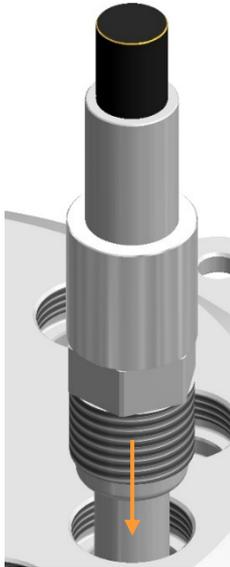
### 8.1.16.2 Mounting a Sensor into a 12 mm Port

For culture vessels with nominal widths of 90 and 145, sensors can be screwed directly into 12 mm/Pg13.5 ports. To do so, proceed as follows:

#### Procedure

1. Slide the O-ring onto the sensor.

## Before Cultivation



2. Insert the sensor into the port.

3. Screw the sensor on its thread into the port by hand.

### 8.1.16.3 Mounting Sensors with Electrode Holder

For the mounting of a sensor in a 12 mm/Pg13.5 port for culture vessels with a nominal width of 115, an electrode holder must be used.

To do so, proceed as follows:

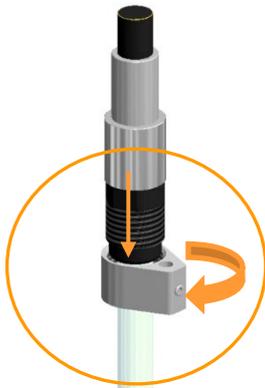
Procedure



1. On the electrode holder, lightly loosen the grub screw in the support guide with the key.

2. Pull the support guide from the guide bar.

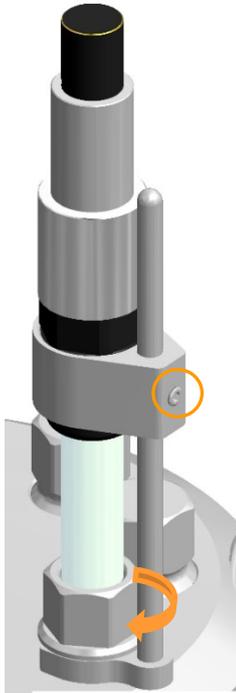
3. Insert the sensor into the support guide and tighten it.



4. Insert the sensor into the hollow screw with the thread pointing in the downward direction.
5. Fit the fork of the guide bar into the groove of the hollow screw.
6. Push the hollow screw and the guide bar together upwards and insert the guide bar into the hole of the support guide.



## Before Cultivation



7. Slide the O-ring onto the sensor and insert the sensor into the port.
8. Adjust the sensor to the desired mounting depth.
9. Screw the sensor on the hollow screw into the port and tighten it.
10. Tighten the grub screw in the support guide with the key.

### 8.1.16.4 Mounting the Antifoam Sensor

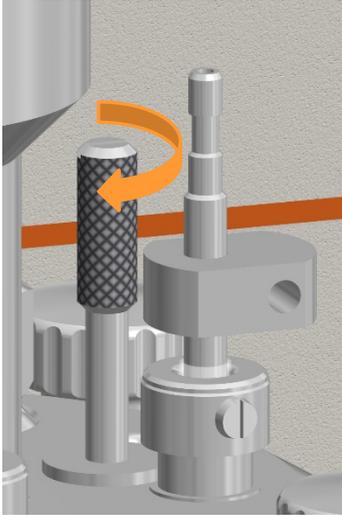
Please note the following points for mounting:

- The antifoam sensor is equipped with transparent insulation that must be intact, as otherwise a continuous signal "Foam/liquid detected" may be generated.
- The sensor head must not touch the clamping adaptor, otherwise a continuous short-circuit is generated, indicating "Foam/liquid detected".
- The clamping adaptor on the sensor must be equipped with an intact O-ring.

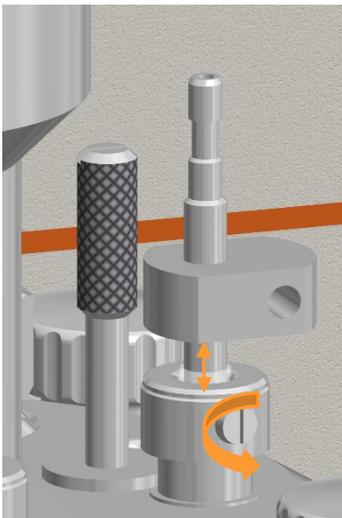
Proceed as follows for mounting:

Procedure

1. Remove the protective cap from the sensor.
2. Insert the sensor into the port.

**Before Cultivation**

3. Fix the clamping adapter with the fastening screw.



4. Loosen the slotted-head screw at the clamping adaptor.
5. Set the desired mounting depth of the sensor carefully.

6. Tighten the slotted-head screw carefully.

**ATTENTION**

If the sensor is fixed too tightly in the clamping adapter, or the mounting depth of the sensor is changed while the screw on the clamping adapter is tightened, the sensor insulation may be damaged.

## Before Cultivation

### 8.1.17 Preparing the Super Safe Sampler



#### INFORMATION

The following figures are for general purposes of comprehension.

In order to prepare the Super Safe Sampler sampling system for autoclaving, proceed as follows:

#### Procedure



1. Attach the hose of the valve group on the dip tube.



2. Secure the hose with a cable tie.
3. Tighten the sample valve carefully by hand in a clockwise direction.  
This ensures that the non-return valve/sample valve screw connection is tight.

**Before Cultivation**

4. Turn the sterile filter carefully by hand in a clockwise direction. This ensures that the non-return valve/sterile filter screw connection is tight.



5. Cover the valve group loosely with aluminium foil.

6. Clamp off the hose on the dip tube.

**8.1.18 Mounting the Sparger Hose and the Inlet Air Filter**

The sparger must be equipped with the hose and inlet air filter before autoclaving.

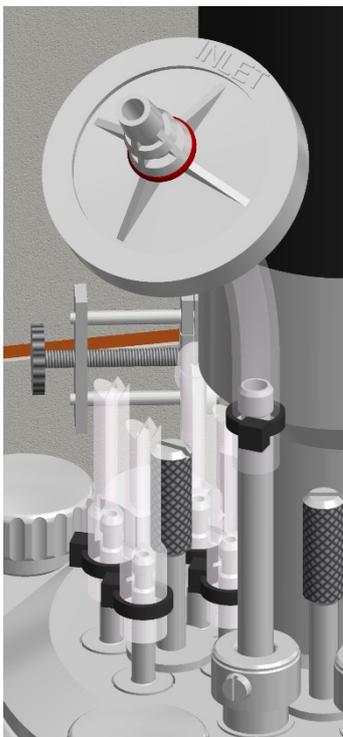
To do so, proceed as follows:

**Procedure**

1. Cut a short piece of hose:
  - Silicone hose  $\varnothing = 5 \times 8$  mm: 1.5 L culture vessel for microorganisms and all vessel sizes for cell culture.
  - Pressure hose transparent,  $\varnothing = 5 \times 10$  mm: 3.0 L and 6.0 L culture vessels for microorganisms.

## Before Cultivation

2. Fit the inlet air filter to the hose piece, fit it in the direction of the air flow to the hose end; the nozzle with the red marking remains exposed:
  - Filter  $\varnothing = 37$  mm: 1.5 L culture vessel for microorganisms and all vessel sizes for cell culture.
  - Filter  $\varnothing = 50$  mm: 3.0 L and 6.0 L culture vessels for microorganisms.



3. Fit the hose to the sparger.

The figure to the left shows an inlet air filter for 1.5 L culture vessels for microorganisms as an example.

4. Secure the ends of the hose with the cable tie.
5. Clamp off the hose with a clamp.
6. Lightly cap the inlet air filter with aluminium foil.

### 8.1.19 Mounting the Hose and Inlet Air Filter for Head Space Gassing

An addition port adapter in the vessel top plate must be equipped with a hose and an inlet air filter for head space gassing before autoclaving.

To do so, proceed as follows:

#### Procedure

1. Cut a short piece of silicone hose ( $\varnothing = 3 \times 6$  mm)

**Before Cultivation**

2. Place the inlet air filter, marked in red,  $\varnothing = 37$  mm, onto the hose end in the direction of the air flow.  
The nozzle with the red INLET marking remains exposed.
3. Place the silicone hose onto the addition port adapter.
4. Secure the ends of the hoses with cable ties.  
If applicable, close unused inlets on the addition port adapter with hose pieces and cable ties.
5. Clamp off the silicon hose with a clamp.
6. Lightly cap the inlet air filter with aluminium foil.

**8.1.20 Preparing the Reagent Bottles, Pumps and Hoses**

250 mL reagent bottles are supplied as standard in the device package. These fit in the reagent bottle holder that is built into the vessel holder. The reagent bottles are equipped with filters for pressure equalisation and hoses of the correct length, and connected to the pump head ex-factory.

**ATTENTION**

Damaged hoses and/or clogged sterile filter may lead to undesired pressure conditions in the reagent bottles.

- Ensure each reagent bottle is equipped with an open pressure equalisation line with a clean and dry filter.
- Only use clean, intact hoses and they are firmly attached.

Below is a detailed description of how reagent bottles are equipped properly and connected to the pumps and culture vessel.

**Connecting the reagent bottles to the pumps and culture vessel**

To connect the reagent bottles with the pumps and the culture vessel, proceed as follows:

Procedure

1. Cut two long silicone hoses ( $\varnothing = 2 \times 6$  mm) per pump/reagent bottle.

**INFORMATION**

The length of the silicone hoses must be selected to ensure that the hose connections between the reagent bottles, pumps and culture vessel do not have any tensions or kinks.

## Before Cultivation



2. Thoroughly rinse the silicone hoses with distilled water.
3. Connect the silicone hoses and pump hoses of the pump heads with hose connectors.

For the **Fill** function:

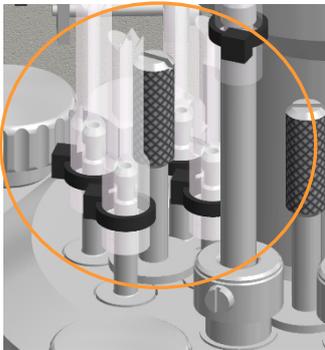
- Right-hand side = suction side = hose line to reagent bottle.
- Left-hand side = pumping side = hose line to culture vessel.

See arrow for direction of rotation.

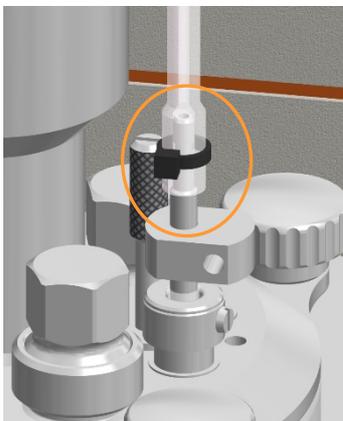
4. Secure with cable ties.

### Connection between pumps and culture vessel

#### Procedure



1. Fit silicone hoses for base, acid and feed to addition port adapter and/or feed needle(s) and secure them with cable ties.



2. Attach the silicone hose of the antifoam pump to the mounted antifoam sensor in the culture vessel and secure it with a cable tie.

**Before Cultivation**

## Procedure

**Connection between reagent bottles and pumps**

1. Ensure that a hose is fitted inside the reagent bottles at the exposed hose connection (without sterile filter); fit one if not:
  - a) the end of the hose does not touch the bottom of the bottle, otherwise the hose may get sucked against the bottom and no longer be able to pump liquid.
  - b) the end of the hose is cut diagonally. In this case the hose end can touch the bottom of the bottle.
2. Label the reagent bottles in accordance with their content.
3. Depending on the application: Fill the reagent bottles with reagents and reclose them with their lid.

**ATTENTION**

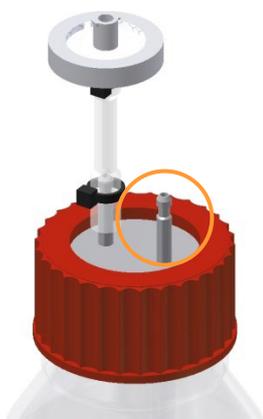
Usage of the highly corrosive hydrochloric acid HCl as reagent leads to damage to components made of stainless steel such as e.g. component parts or the top plate.

Use only non-corrosive acids, e.g. phosphoric acid, instead.

**INFORMATION**

Fill reagent bottles with heat-resistant reagents only. Sterilise non-heat-resistant feed solution separately and only transfer it to the reagent bottle after sterilising.

4. Place the reagent bottles in reagent bottle and pump holders.
5. Attach the correct silicone hoses to available hose connections of each reagent bottle and secure them with cable ties.



## Before Cultivation

6. Close silicone hoses with clamps as close as possible to the hose connections of the reagent bottles to ensure that no reagent can flow into the culture vessel.
7. Ensure that:
  - each reagent bottle is connected with the appropriate pump according to its contents. (Base to base pump, etc.)
  - filters are clean and dry; short hose line is open.
8. Cap the filter loosely with aluminium foil.

### 8.1.21 Sterile Hose Connections

If additional vessels are needed and these can only be connected to the culture vessel after autoclaving, such as vessels for the inoculum or bottles for sampling etc., rapid couplings (male/female), sterile connectors or – if weldable hoses are used – a hose welding device can be used to form a sterile connection.

The connection pieces must be fitted to the appropriate hoses before autoclaving. Rapid couplings are connected after autoclaving in a sterile workbench. Sterile connectors and hose welding devices allow sterile connecting without a sterile workbench.

### 8.1.22 Setting the Pumps

If the pumps are not used with the default settings, we recommend that the appropriate settings are now made on the operating panel.

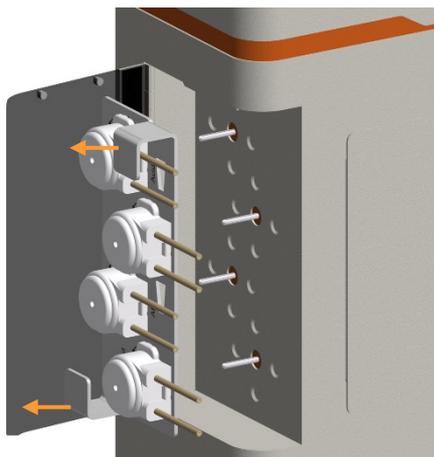
It is possible, for example, to estimate and display the volume (in mL) that has been pumped since the Batch (process) started. To this end, the diameter of the hose used must be selected.

For details on the pumps and the setting options, see the main chapter "Operation", the "PUMPS parameter group" chapter and the associated chapters.

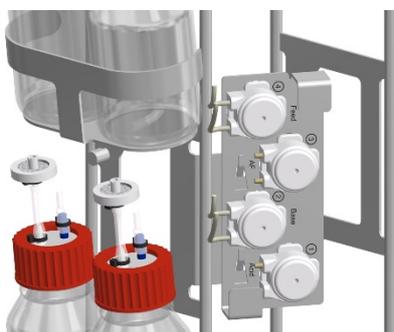
### 8.1.23 Removing the Pump Heads

To remove the pump heads from the basic unit, proceed as follows:

Procedure



1. Swing open the pump cover.
2. Remove the mounting plate with the pump heads from the drive shafts by holding the two handles.



3. Place the mounting plate with the pump heads onto the pump holder on the vessel holder.

### 8.1.24 Fitting the Cone Plug for Drive Hub

In order to prevent the penetration of condensation water into the drive hub during autoclaving, the cone plug provided in the starter set must be fitted.



#### ATTENTION

Risk of loss of property due to penetration of condensation water into the drive hub!

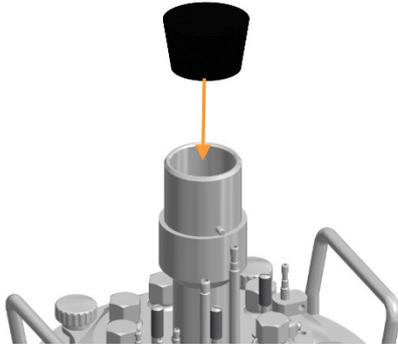
Always autoclave the culture vessel with the cone plug fitted to the drive hub!

## Before Cultivation

Proceed as follows:

Procedure

1. Plug the cone plug into the opening of the drive hub.



### 8.1.25 Checklist Before Autoclaving

Check and ensure the following items before autoclaving:

#### Culture vessel

All necessary O-rings are fitted.

All unused ports are closed with blanking plugs

Connection for inoculation is equipped with septum, septum collar and blanking plug

**Drive hub** is equipped with **cone plug**.

There is liquid in the culture vessel (autoclavable medium or approx. 10 mL water per litre working volume).

#### Reagent bottles, hoses and pumps

Reagent bottles are exclusively filled with autoclavable reagents, correctly labelled and connected with the culture vessel and the pump heads via hoses.

Reagent bottles are equipped with filters for pressure equalisation

Reagent bottles are placed in reagent bottle holders and pump heads are placed on the pump holder with a mounting plate.

#### Sampling System Super Safe Sampler

The valve group is connected to the dip tube in the culture vessel by means of a hose.

The valve group is lightly capped with aluminium foil.

**Before Cultivation****Sparger, Head space gassing, exit gas cooler**

The sparger is equipped with a hose and an inlet air filter.

Version for cell culture: addition port adapter for head space gassing is equipped with hose and inlet air filter.

The exit gas cooler is equipped with a new securely fastened exit gas filter.

**Filters & hoses**

All filters are clean, dry and lightly capped with aluminium foil.

There are no open hose ends.

All hose transition points are secured with an autoclavable cable tie or hose clamp to prevent them from slipping.

Hoses on the reagent bottles, for sampling and the gassing system are clamped off with clamps.

The exit gas hose is **NOT** clamped off.

The hoses are undamaged; the hose lines show no kinks and are not able to kink.

**Sensors**

All sensors required are mounted and, if necessary, calibrated.

The antifoam sensor is mounted, set for the correct mounting depth, and connected to the correct reagent bottle.

The temperature sensor of the autoclave is inserted into the immersion pocket for the temperature sensor of the culture vessel.

**Do not** cover the pH and pO<sub>2</sub> sensors with aluminium foil!

**8.1.26 Autoclaving**

Before cultivation starts, the culture vessel is autoclaved in accordance with the application in question. The culture vessel can be autoclaved with or without medium.

Adhere to the following:

- Never autoclave the culture vessel dry; see also the chapter "Moistening/Filling the Culture Vessel".

**INFORMATION**

Development of steam is not possible when autoclaving a completely empty and dry culture vessel. Successful sterilisation is not guaranteed.

Ensure that there is liquid in the culture vessel (approx. 10 mL of water per litre of total volume).

## Before Cultivation

- If necessary, pump off any remaining water after autoclaving by means of the dip tube.
- Sterilise all liquid, heat-instable components separately and add them after autoclaving.
- If the medium is autoclaved in the culture vessel, you may then need to add sterile water to make up the volume.

When transporting the culture vessel to/from the autoclave, note the following:

- Always transport the culture vessel in the vessel holder.
- Always transport the culture vessel to/from the autoclave in pairs and use suitable auxiliary equipment when transporting the culture vessel.



### WARNING

Depending on the design, accessories and fill level, the culture vessel may be too heavy to be carried by one person alone.

Proceed as follows to autoclave the culture vessel:

#### Procedure

1. Place the culture vessel into the autoclave.
2. Ensure that the culture vessel and the accessories do not touch the inner wall of the autoclave.
3. Ensure that the exit gas filter is open.
4. Insert the temperature sensor of the autoclave into the immersion pocket for the temperature sensor.
5. Select the program for liquids.
6. Autoclave the culture vessel in accordance with the operating manual of the autoclave manufacturer.

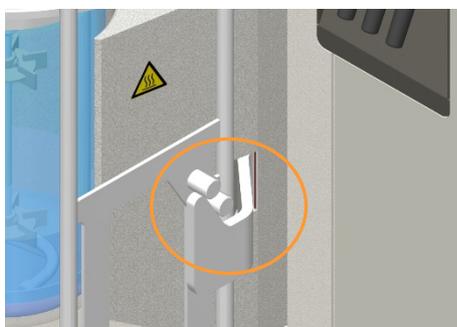
## 8.2 Connecting the Culture Vessel and Preparing the Cultivation

As soon as the culture vessel with the accessories has cooled sufficiently, it can be hung up within the basic unit and the various cable and tube connections between the basic unit and the culture vessel can be established.

### 8.2.1 Hang the Culture Vessel in Place and Fit the Pump Heads

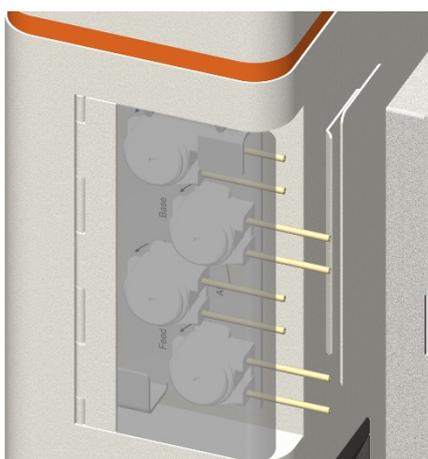
Proceed as follows:

Procedure



1. Hang the vessel holder into place on the two hooks on the thermal block adapter.

2. Pull off the mounting plate with the pump heads from the pump holder
3. If necessary, flip up the pump cover plate.



4. Plug the mounting plate with the pump heads onto the pump motor drive shafts and close the cover plate.

## Before Cultivation

### 8.2.2 Filling the Reagent Hoses

In order to prepare the reagent hoses for operation, you must use the **Fill** function on the operating panel to fill up with reagent via the correct pump.

Before filling, remove the clamps from the reagent hoses.



#### WARNING

When using heavily corrosive reagents (acids and bases), it is particularly important only to use suitable and undamaged hoses. They must also be securely fastened. Furthermore, the exit gas filter must not be blocked. This ensures that no pressure builds up and no reagent escapes due to burst hoses.

When filling, ensure that no reagent escapes into the culture vessel, if possible.

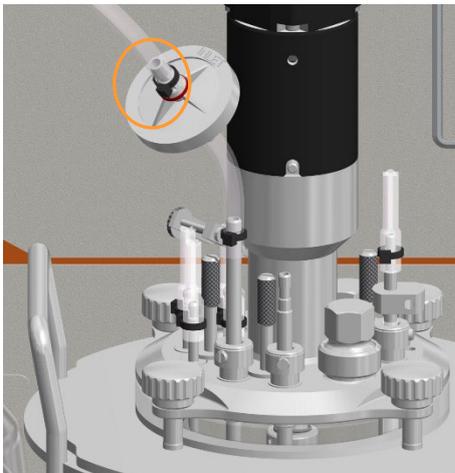
For details on filling, see the main chapter "Operation", chapter "Parameter Group PUMPS".

### 8.2.3 Connecting the Gassing

To connect the sparger and the addition port adapter (head space gassing for cell culture version) to the gassing, proceed as follows:

Procedure

1. Remove the aluminium foil from the inlet air filter.
2. Insert the gassing hose of the basic unit to the inlet air filter of the sparger and secure it in place with a cable tie.



The figure to the left shows an inlet air filter for 1.5 L culture vessels for microorganisms as an example.

3. Remove the hose clamp.

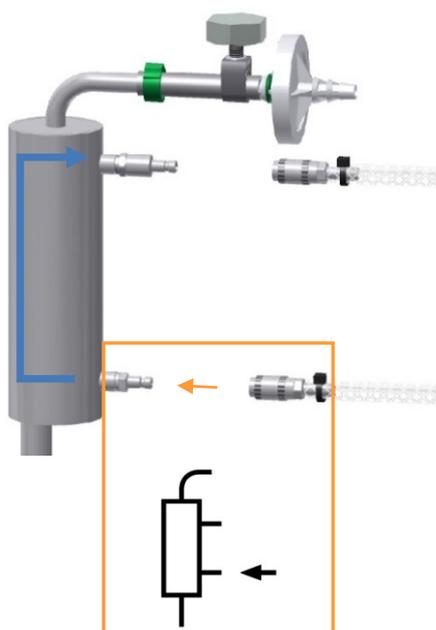
4. Head space gassing: connect the gassing hose from the basic unit to the inlet air filter on the addition port adapter and secure it with a cable tie.
5. Remove the hose clamp.

### 8.2.4 Connecting the Exit Gas Cooler

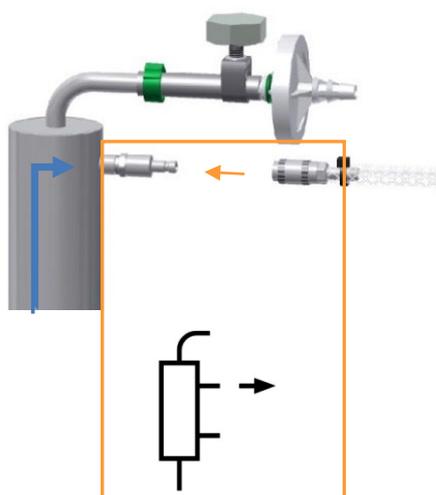
To connect the exit gas cooler to the basic unit, proceed as follows:

Procedure

1. Remove the aluminium foil from the exit gas filter.
2. Insert the rapid coupling of the water **inlet** hose – note the symbol on the basic unit - onto the **lower** connection nozzle on the exit gas cooler.



3. Insert the rapid coupling of the water **outlet** hose – note the symbol on the basic unit - onto the **upper** connection nozzle on the exit gas cooler.



## Before Cultivation



### INFORMATION

The exit gas cooler only works when the temperature control system is switched on (*Temperature ON* parameter)

### 8.2.5 Connecting the Cold Finger

To connect the optional cold finger to the basic unit, connect the hoses for water inlet and return via the rapid couplings, taking the water flow direction into account.

For details on the cold finger, refer to main chapter "Accessories", chapter "Cold Finger".

### 8.2.6 Coupling the Motor

For routine operation, it is not necessary to plug in and unplug the motor cable. The motor connected during installation is only coupled before cultivation.



### ATTENTION

If the motor cable is connected to or disconnected from the motor while the device is switched on, there is a risk of a short circuit that could damage the control electronics.

For details about connecting the motor cable, see the main chapter "Installation and Initial Operation", chapter "Connecting the Motor Cable".

To couple the motor, proceed as follows:

Procedure



1. Place the motor onto the drive hub with the groove aligned with the pin on the drive hub.  
The motor is held in its position.

### 8.2.7 Filling the Culture Vessel

Depending on the application, the vessel can be filled after autoclaving. To prevent foam formation during filling, add the medium via a dip tube.

To do so, proceed as follows:

#### Procedure

1. Sterilise the medium separately.
2. If necessary, pump off any water that remains in the culture vessel.
3. Establish a sterile hose connection between the culture vessel and the medium container.
4. Pump the desired quantity of medium into the culture vessel.
5. Clamp off the medium hose; if necessary, apply a welded seal.
6. Disconnect the medium container from the culture vessel; if necessary, retain it as a harvest or waste container.



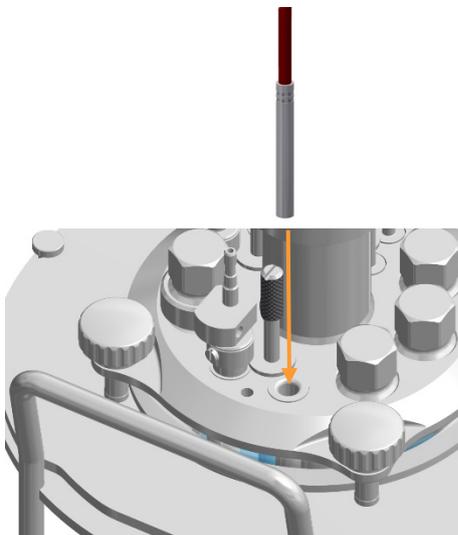
#### INFORMATION

If the stirrer is turning on the surface of the medium, foam will be formed. For this reason, only switch on the stirrer if it is fully covered by medium.

### 8.2.8 Connecting the Temperature Sensor (Pt100)

The temperature sensor is not in direct contact with the medium.

#### Procedure



1. Insert the sensor into the immersion pocket in the vessel top plate as far as it will go.

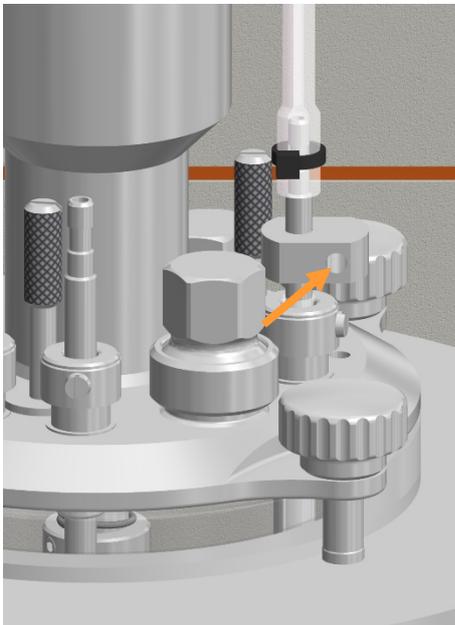
## Before Cultivation

### 8.2.9 Connecting the Antifoam Sensor

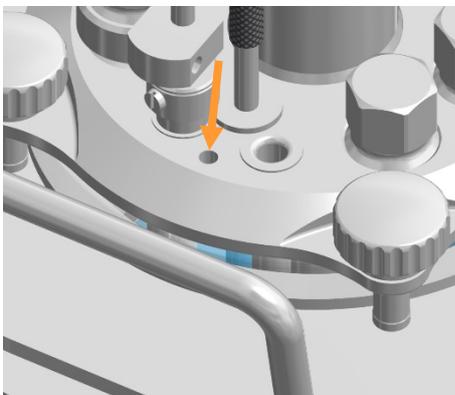


To connect the antifoam sensor, the two banana connectors of the sensor cable must be inserted as follows:

#### Procedure



1. Insert the red banana plug into the connector on the sensor head.

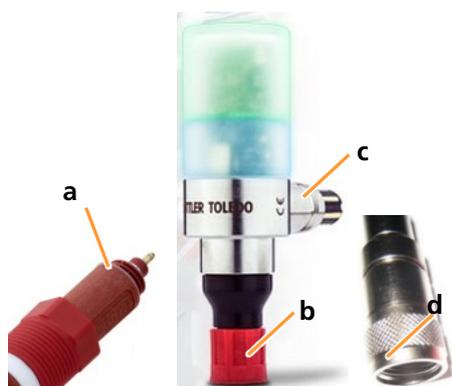


2. Insert the black banana plug into the earth connection in the top plate.

**Before Cultivation**

**8.2.10 Connecting the pH Sensor**

The two available pH measurement systems have the following sensor and cable connections:



METTLER digital Type InPro 3253i	Sensor head connection (a)	ISM
	Cable bushing (d)	VP8
Head transmitter M100	Plug connection for sensor (b)	
	Plug connection for cable (c)	



HAMILTON digital Type Easyferm Plus ARC	Sensor head connection (a)	VP8
	Cable bushing (b)	VP8

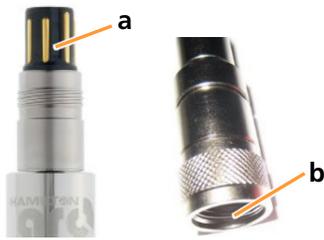
**8.2.11 Connecting the pO<sub>2</sub> Sensor**

Both available pO<sub>2</sub> measurement systems have the following sensor and cable connections:



METTLER digital Type InPro6860i	Sensor head connection (a)	VP8
	Cable bushing (b)	VP8

## Before Cultivation



HAMILTON digital Type Visiform DO ARC	Sensor head connection (a)	VP8
	Cable bushing (b)	VP8

### 8.2.12 Calibrating the pO<sub>2</sub> Sensor

A 1-point calibration to 100 % is usually sufficient for exact measurement and should be carried out before each cultivation. If required, a 2-point calibration to 100 % and 0 is also possible.

For more details on the calibration refer to main chapter "Operation", chapter "Calibrating the pO<sub>2</sub> Sensor".

### 8.2.13 Checking the Hoses and Hose Connections

Check and ensure the following items before each cultivation:

- Hoses show no kinks and are not able to kink.
- Hoses are undamaged and show no weaknesses.
- Gas hoses and connections do not show any leaks.
- Hose lines are as short as possible.
- Hoses are secured with cable ties and/or hose clamps.
- Only the pressure hoses supplied by the device manufacturer are connected as supply lines (water, gas) between the in-house connections and the device.

## 9 Cultivation

The following chapters describe the work necessary for the performance of and after the completion of a cultivation, before the culture vessel with accessories is thoroughly cleaned and then prepared for another cultivation.

This essentially comprises:

- Preparing the medium (starting a Batch)
- Sampling
- Inoculation
- Harvest
- (Stopping the Batch), if necessary emptying the vessel
- Autoclaving the culture vessel and accessories

The requirement for the first item is that the culture vessel and accessories are autoclaved, cooled and connected to the basic unit. All cable and hose connections between the device and the culture vessel, including the reagent bottles, are present, pump heads are mounted and the reagent hoses are filled. Depending on the user specifications, the pO<sub>2</sub> sensor is already calibrated.

### 9.1 Preparing the Medium

Before the first sampling, which usually takes place as a 'zero sample' before inoculation, and before the inoculation itself, the medium must be warmed to the desired temperature. If necessary, the pO<sub>2</sub> concentration and the pH are set. The time required for this depends on the working volume.

Set and activate the desired setpoint of the parameter in question on the operating panel, and start the Batch (process).

Depending on the specifications defined by the user, the pO<sub>2</sub> sensor is calibrated either before the vessel is filled with medium or afterwards, in the prepared medium.

## Cultivation



### CAUTION

If pressure equalisation does not take place via a top plate opening or the mounted exit gas cooler, overpressure in the culture vessel may occur during cultivation as a result of warming, gassing or fermentation processes.

- Exit gas line - hose at the exit gas cooler with secured exit gas filter - ALWAYS keep open.
- Only use clean and dry exit gas filters.

For details on operation, see the corresponding chapter in the main chapter "Operation".

For more details on the calibration refer to main chapter "Operation", chapter "Calibrating the pO<sub>2</sub> Sensor".

## 9.2 Sampling

Samples are taken from the culture vessel to gain material for offline analysis.



### INFORMATION

Using **SAMPLE NOW** on the operating panel, the sampling can be logged in the electronic logbook and assigned a sample ID. For more details, see main chapter "Operation".

The method of sampling can vary due to the different analyses carried out by the operator.

The sampling procedure using the standard sampling system, Super Safe Sampler, is described below.

Before starting, observe the following:

**WARNING**

Culture solution could emerge from the vessel if the sample valve mechanically fails. This could lead to serious health risks in the event of applications with pathogenic organisms.

- When working with pathogenic organisms, always additionally clamp off the sampling hose with a metal (!) clamp.
- Only remove the clamp when sampling.
- Reattach the clamp before removing the syringe from the sample valve.

**WARNING**

Loose screws at components could lead to the penetration of unsterile air or contamination of the environment.

Before and after autoclaving: Check that all screws are tightly screwed in and, if necessary, tighten them manually

If the sample is to be further aseptically processed, use a sterile syringe and sterile closing caps.

For details, see the main chapter "Accessories", chapter "Sampling System Super Safe Sampler", section "Aseptic Sampling".

Proceed as follows:

## Procedure

1. Check that all screw connections of the valve group are tightly screwed in. If necessary, gently tighten the screw connections with two fingers.
2. Remove the clamp from the sampling hose.
3. If present: Remove the closing caps.
4. If desired: Disinfect the sample valve.

## Cultivation



5. Screw open the Luer-Lock syringe on the sample valve.



6. Pull back the syringe plunger to remove the desired sampling volume.

If the dip tube was rinsed with air, air is sucked in first. Remove it as follows:

- a) Unscrew the syringe from the valve.
- b) Hold the syringe with the plunger downwards so that the medium remains in the syringe.
- c) Push the air out of the syringe.
- d) Screw the syringe onto the sample valve.
- e) Draw in again.

7. Attach the clamp to the sampling hose.

### Rinsing the dip tube with sterile air

The dip tube and its sampling hose can be filled with sterile air after taking a sample.

**INFORMATION**

Only use a clean and dry syringe to avoid blocking the sterile filter. This syringe can be reused as often as desired, since air is provided via a sterile filter.

To do so, proceed as follows:

Procedure:



1. Insert the syringe onto the hose at the sterile filter and push air through.

The remaining liquid in the hose and in the dip tube is pushed back into the vessel.

2. Remove the syringe from the sterile filter to fill it with air again.
3. Repeat steps 1 and 2 as many times as necessary until bubbles rise out of the dip tube.

**Removing residual fluid**

To remove residual fluid from the system, proceed as follows:

Procedure:



1. Hold the syringe with sample downwards, pull back the plunger. This removes all but a few  $\mu\text{L}$  of the residual fluid.

## Cultivation



2. Hold the sample valve with one hand; unscrew the syringe with the other.

3. If desired: Place the closing caps on the sample valve and on the syringe with the sample.

### 9.3 Inoculation

Check and ensure the following items before inoculation:

- Medium has been filled.
- Heat-labile, separately sterilised substances are present.
- The reagent bottles are connected to the pumps and the culture vessel and are filled with reagents and nutrient solution enough for the duration of the entire cultivation.
- The hoses of the reagent bottles are filled.
- The correct operating temperature has been reached.
- The required stirring speed is set.
- The sensors are calibrated, and the control is set correctly (maybe not active yet).
- All clamps have been removed (except for sampling system).
- Utensils for the inoculation and vessels with inoculum are ready.

#### Methods

There are a number of ways to add medium or inoculum before and during cultivation:

- In a small volume, with the syringe via the septum
- Via the addition port adapter from the reagent bottle (a sterile hose connection is required for this method).

- Via the dip tube from the reagent bottle (a sterile hose connection is required for this method).

These methods are described below.

The implements for inoculation with a syringe are standard accessories for the device. This inoculation method is particularly suitable for all vessel sizes of the device.

### 9.3.1 Inoculation with a Syringe

Proceed as follows for the inoculation:

Procedure

1. Fill the syringe with the required amount of inoculum.
2. Unscrew the blanking plug from the septum collar.  
As a possible additional protection against contamination:  
Before piercing, drop a few drops of ethanol (70 %) on the septum.
3. Pierce the septum and inject the inoculum.
4. Remove the needle from the septum and close the septum collar with a blanking plug.

### 9.3.2 Inoculation Using Dip Tube / Addition Port Adapter

Proceed as follows during inoculation:

Procedure

1. Fill the inoculum under sterile conditions into the prepared container.
2. Create a sterile hose connection with the dip tube/addition port adapter.
3. Transfer the desired volume of inoculum into the culture vessel. Pump it, if necessary.
4. Clamp off the hose by means of a clamp, weld it if necessary.

## 9.4 Harvest

The culture can be harvested at the end of the cultivation. To prevent possible sedimentation from the culture, the stirrer can be switched on during harvesting. If necessary, activate gassing for sensitive cultures. However, all other parameters should be switched off, provided there are no other specifications for the user.

## Cultivation

The following possibilities exist for the harvest:

- Transfer to another vessel: transfer the contents of the vessel to another container in a laminar flow cabinet.
- Pump-down via a sterile hose connection: to do so, proceed as follows:

### Procedure

1. Make a sterile connection between the hose at the dip tube for harvest and the new vessel.
2. Connect the hose to one of the pumps on the device or to an external pump.
3. Pump the desired amount of culture into the new vessel.



### INFORMATION

Only switch on the stirrer if it is fully covered by medium, as foam otherwise forms.

4. Switch off all parameters at the operating panel and stop the Batch (process) at the operating panel.



### INFORMATION

Always stop the running Batch (process) on the operating panel. If it is stopped by pressing the power switch, it is akin to a power interruption. This means that when it is switched on again, the previous settings are adopted and the Batch continues running where it was interrupted. This also applies, if the Batch is controlled via eve®, the platform software for bioprocesses.

## 9.5 Emptying the Culture Vessel

Depending on the user specifications, the culture vessel can be emptied either before or after autoclaving.

A previously emptied and culture vessel filled only with water for autoclaving is easier to clean afterwards.

For emptying the culture vessel, the same options as for harvesting are available. For more information, see chapter "Harvest".

If the culture will not be used further, it must be inactivated according to the current in-house instructions (e.g. by autoclaving or by lowering the pH value), and subsequently disposed of in an environmentally sound manner according to the local regulations.

## 9.6 Emptying the Reagent Hoses

Before autoclaving the culture vessel with accessories, all reagent hoses must be completely emptied using the corresponding pump. This can either be done manually or time-controlled at the operating panel.



### ATTENTION

Residues of acids and alkalis in the reagent hoses during autoclaving can damage the pump heads.

- Completely empty all reagent hoses before autoclaving.
- Thoroughly rinse the reagent hoses with water after emptying.



### INFORMATION

If feed needle(s) are used instead of addition port adapters, the vessel contents are simultaneously pumped back into the reagent bottle while emptying the hoses, if the vessel has not been previously emptied.

## 9.7 Switching off the Device

When the harvest is finished or the culture vessel has been emptied and the reagent hoses are also empty, the device can be switched off.

Proceed as follows:

Procedure

1. Ensure that the Batch (process) has been stopped. If necessary, stop it using **Stop Batch**.



### INFORMATION

Always stop the running Batch (process) on the operating panel. If it is stopped by pressing the power switch, it is akin to a power interruption. This means that when it is switched on again, the previous settings are adopted and the Batch continues running where it was interrupted. This also applies, if the Batch is controlled via eve®, the platform software for bioprocesses.

## Cultivation

2. Press the power switch to switch off the device.
3. Let the motor cool down (device version for microorganisms).

### CAUTION

Risk of minor burns if the motor, which heats up during operation, is touched!

4. Close the supply lines (water, gas).
5. Autoclave the vessel, component parts and accessories as per the user-specific specifications and then clean them.

## 9.8 Autoclaving the Culture Vessel After Cultivation

After emptying the culture vessel and before cleaning, the culture vessel must be autoclaved with all accessories.

When doing so, do not autoclave the culture vessel when completely dry and observe the same safety regulations as when autoclaving before cultivation.

Before starting, ensure:

- There is liquid in the culture vessel (autoclavable medium or approx. 10 mL water per litre working volume).
- Reagents and feed solution have been pumped back out of the hoses.
- The device is switched off.
- The motor has cooled down (version for microorganisms).

Proceed as follows to prepare the culture vessel and accessories for autoclaving after cultivation:

### Procedure

1. Clamp off the hoses of the reagent bottles.
2. Clamp off the hose of the sparger, and where applicable, the hose for head space gassing.
3. Remove all cable and hose connections between the basic unit and the culture vessel:
  - a) Uncouple the motor and place it to the side.
  - b) Unplug the sensor cables.
  - c) Pull the temperature sensor out of the immersion pocket.

- d) Disconnect the water inlet and water outlet hoses from the exit gas cooler.
- e) Remove the gassing hose (emerging from basic unit) from the inlet air filter on the sparger and from the inlet air filter on addition port adapter (head space gassing for cell culture).

4. Lightly cover all filters with aluminium foil.

**ATTENTION**

**Do not** cover the pH and pO<sub>2</sub> sensors with aluminium foil!

5. Fit the cone plug into the opening of the drive hub.

**ATTENTION**

Risk of loss of property due penetration of condensation water into the drive hub!

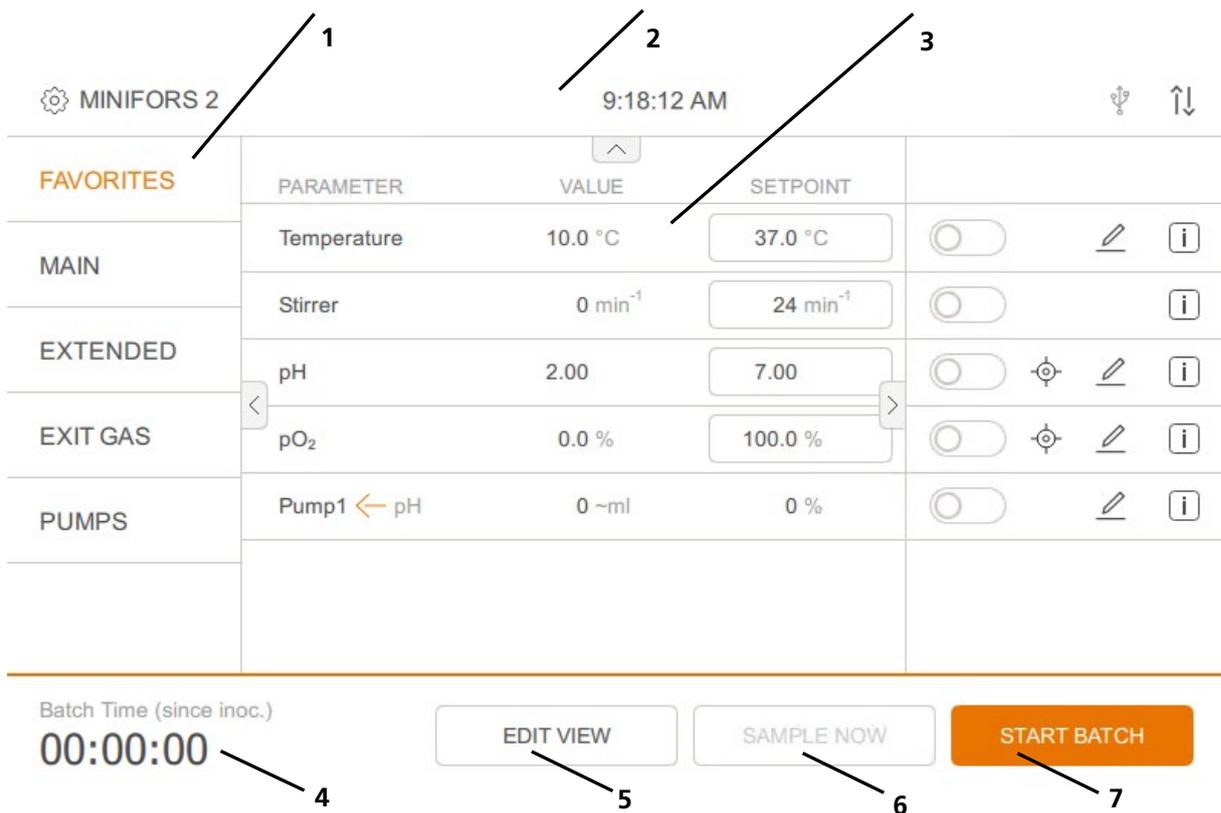
Always autoclave the culture vessel with the cone plug fitted to the drive hub!

- 6. Open the pump cover.
- 7. Remove the mounting plate with pump heads from the drive shafts on the basic unit and place on the pump holder.
- 8. Check and ensure that the exit gas filter is free and dry and the exit gas hose is **OPEN**.
- 9. Insert the temperature sensor of the autoclave into the immersion pocket on the culture vessel and autoclave the culture vessel.

**Operation**

**10 Operation**

**10.1 Screen Areas, Menu Navigation and Control Elements**



- |                                                                                                                                                                                                                      |                                                                                                                                                                                                       |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>1 Left side with selection menus for system settings or parameter groups</p> <p>2 Header with status displays</p> <p>3 Main screen</p> <p>4 Display of the Batch time (since inoculation) and possible alarms</p> | <p>5 Button for the selection of the parameter display</p> <p>6 Button for time stamp for sampling</p> <p>7 Buttons with changing function for Batch start, inoculation time stamp and Batch stop</p> |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Header status display**

The following symbols and displays are in the header:



- *Settings* symbol: to switch between menu selection for system settings and parameter groups

13:34:58

- Display of the current time



- Display for connected USB stick



- Display for an active connection to SCADA software



**Alarm display**

If alarms occur (device) equipment alarm or parameter alarm), they are signalled by a red exclamation mark highlighted in white on a red background. Pressing the symbol or swiping upwards opens the alarm menu.

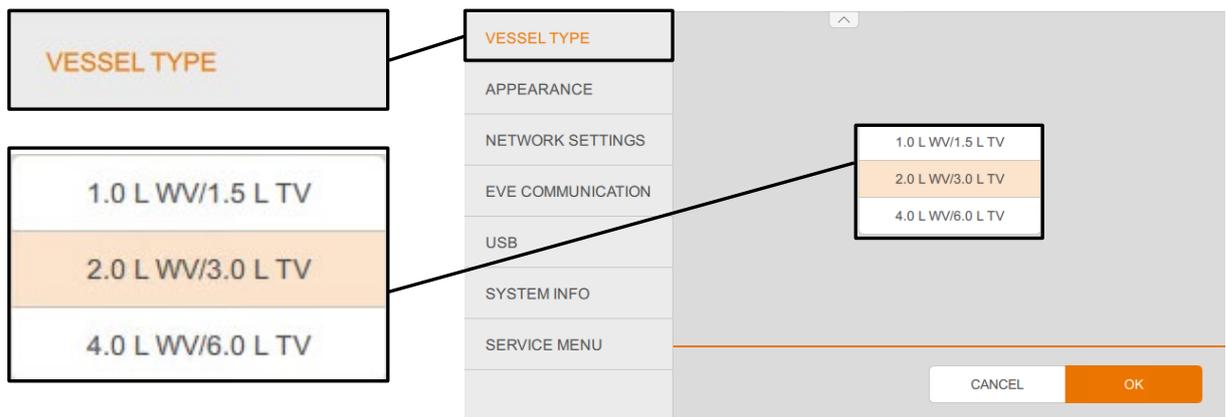
For details, see the “Alarms – Equipment Alarm Menu” and “Parameter Alarms” chapters.

## Operation

### 10.1.1 Main Screen

Depending on the menu selected on the left side of the screen, the main screen displays the following:

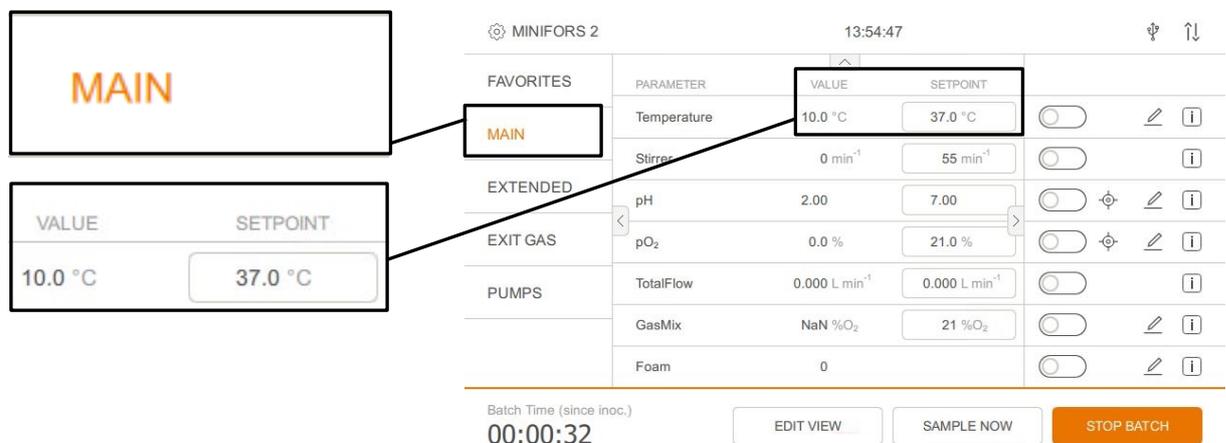
- a) **Menus for system settings**, such as the *VESSEL TYPE* menu for setting the vessel size.



Depending on the menu selected, the **CANCEL** and **OK** buttons, or only the **OK** button, are available in the menu footer:

- **OK** saves the changes and closes the menu.
- **CANCEL** closes the menu without making any changes.

- b) **Parameter group** with parameter actual values and setpoints, such as *MAIN* parameter group with actual values in the *VALUE* column and input fields for setpoints in the *SETPOINT* column



MAIN



MAIN

All menus and parameter groups can be selected by pressing them. The selected menu or parameter group is highlighted with a change of colour in the menu/group text from black to orange.

Example to the left: *MAIN* parameter group



The arrow buttons at the edge of the main screen can be used to show or hide parts of the menu and display.

PARAMETER	VALUE	SETPOINT
Temperature	10.0 °C	37.0 °C
Stirrer	0 min <sup>-1</sup>	55 min <sup>-1</sup>
pH	2.00	7.00
pO <sub>2</sub>	0.0 %	21.0 %
TotalFlow	0.000 L min <sup>-1</sup>	0.000 L min <sup>-1</sup>
GasMix	NaN %O <sub>2</sub>	21 %O <sub>2</sub>
Foam	0	

The figure to the left shows the example of the menu with parameter options, which becomes visible after pressing the arrow button on the right-hand edge of the screen (figure above).



**INFORMATION**

Instead of using the arrow buttons, swiping movements to the left, right, upwards or downwards on the screen can also change the display.

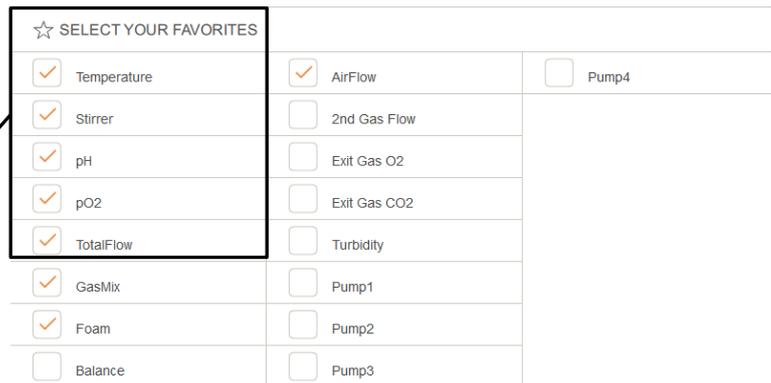
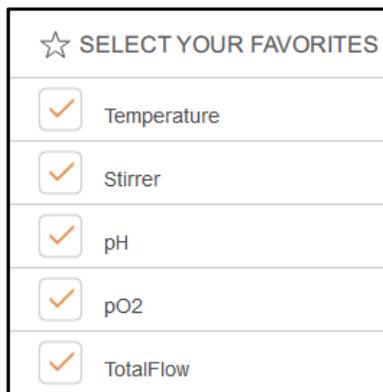
## Operation

### 10.1.2 EDIT VIEW



**EDIT VIEW** opens a menu with all available parameters.

Here, up to 8 parameters can be selected to appear in the *FAVORITES* parameter group by checking the check boxes.



- **OK** confirms the selection and closes the menu.
- **CANCEL** closes the menu without making any changes.

### 10.1.3 START BATCH / INOCULATE / STOP BATCH



Pressing the **START BATCH** button starts the preparation phase for the Batch (bioprocess). The controller is activated. The current parameter settings are simultaneously logged in a log file, and recording of the actual values begins.

**i** **INFORMATION**

Log files can be exported on a USB stick.



The button now changes function to **INOCULATE**. In this process phase, the parameters can be activated manually and individually.

#### INOCULATION

Do you really want to inoculate the Batch?



When all preparations are finished, inoculation can take place. After pressing **INOCULATE**, a dialogue appears asking for confirmation that inoculation takes place.

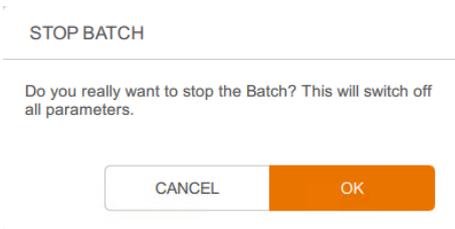
This is signalled by pressing **INOCULATE**. This means that this time corresponds to  $t = 0$  of the Batch time.

Batch Time (since inoc.)  
**00:00:03**

After confirming by pressing **OK**, the *Batch-Time* is started.



The button now changes function to **STOP BATCH**.



After pressing **STOP BATCH**, a dialogue appears asking for confirmation that the Batch is to be stopped, as well as the notice that doing so switches all parameters off.

- **CANCEL** cancels the stop procedure without making any changes.
- **OK** finishes the batch, all parameters are deactivated and the controller is deactivated. Recording of actual values is ended, the button changes function back to **START BATCH**.



**INFORMATION**

*Batch time* remains visible until a new Batch is started or the device is switched off using the main switch.

## Operation

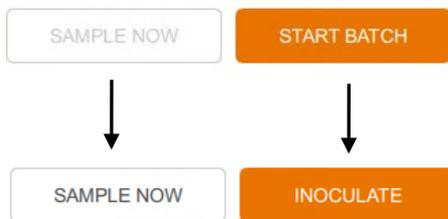
### 10.1.4 SAMPLE NOW



If a sample is removed from the culture vessel by hand, this can be signalled to the bioreactor by pressing **SAMPLE NOW**. This logs the sampling, and is visible in the log files for the batch. For details, see the "USB Data Export and Import from a USB Stick" chapter.

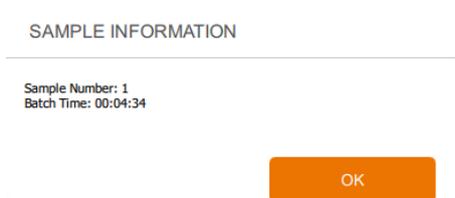
If the bioreactor is connected with the bioprocess platform software *eve*<sup>®</sup>, an *offline sample* is automatically created there.

For details on the procedure for process-suitable sampling, see the main chapter "Cultivation", chapter "Sampling".



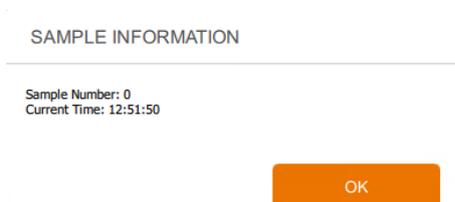
The **SAMPLE NOW** button only becomes functional after pressing **START BATCH**. This means that it can only be used during a batch.

**SAMPLE NOW** generates consecutive numbers for all samples and logs them with the batch time since inoculation as the time stamp.



This means that an Information dialogue appears and displays how long the batch has been running since inoculation (*Batch Time...*), and how many samples have been taken (*Sample Number...*), or how many times **SAMPLE NOW** has been pressed since **START BATCH** was pressed.

**OK** closes the dialogue.

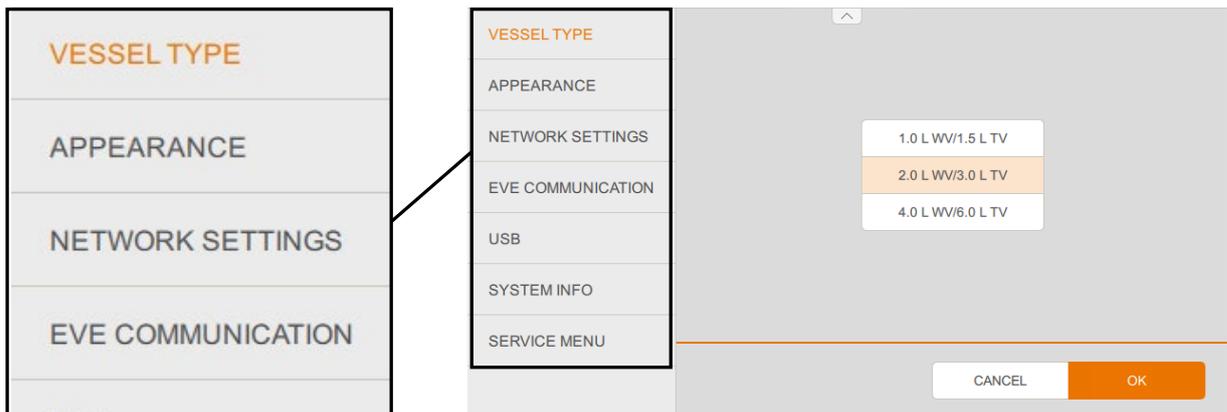


**i** INFORMATION

If sampling takes place before inoculation, the current time is logged instead of the Batch time since inoculation. There is no numbering of the samples taken before inoculation.

## 10.2 Menus for System Settings

There are seven menus for system settings, of which five are available for the operator.



- **VESSEL TYPE:** Selection of the culture vessel used
- **APPEARANCE:** Display settings, including language and date/time
- **NETWORK SETTINGS:** Network configuration
- **EVE COMMUNICATION:** Configuration of the OPC UA server for communication with the bioprocess platform software eve® by the device manufacturer.
- **USB:** Export files on a USB stick or load updates and additional packages from a USB stick.
- **SYSTEM INFO:** Information such as e.g. system and controller version, system uptime etc.
- **SERVICE MENU:** Functions for authorised service partners of the device manufacturer, only accessible with the relevant password



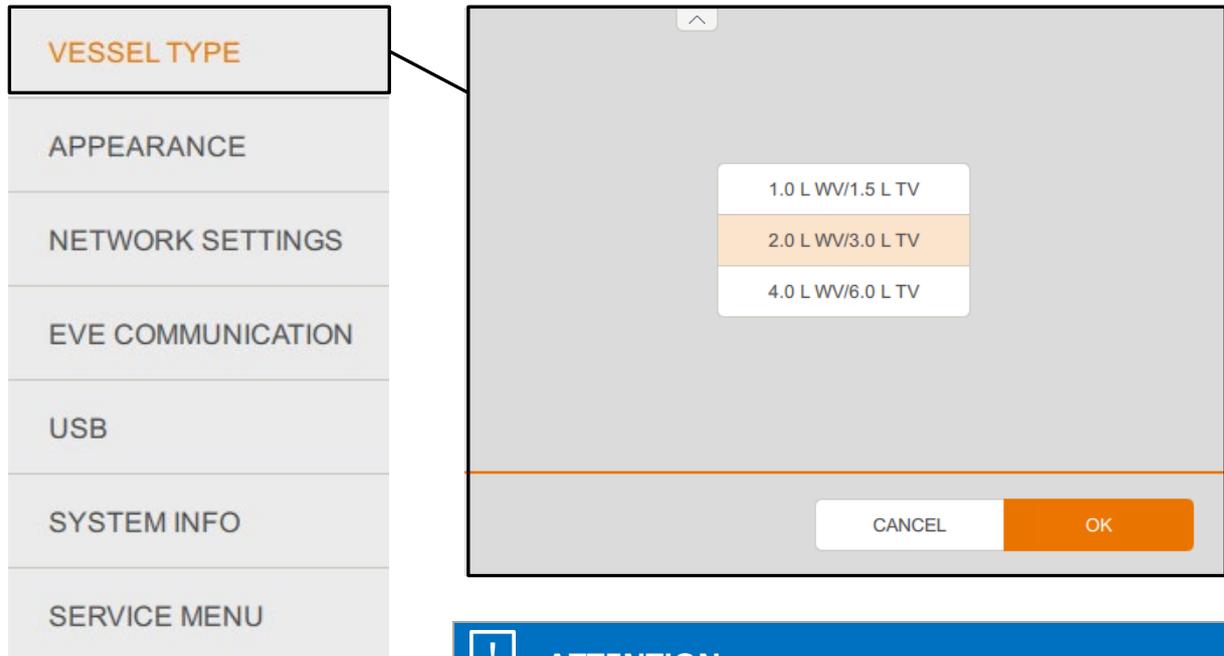
### INFORMATION

Depending on the menu selected, the **CANCEL** (leave the menu without changes) and **OK** (save changes and leave the menu) buttons, or only the **OK** button, are available in the menu footer.

## Operation

### 10.2.1 VESSEL TYPE – Selecting a Culture Vessel

The culture vessel used is set in the *VESSEL TYPE* menu. There are three culture vessel sizes.

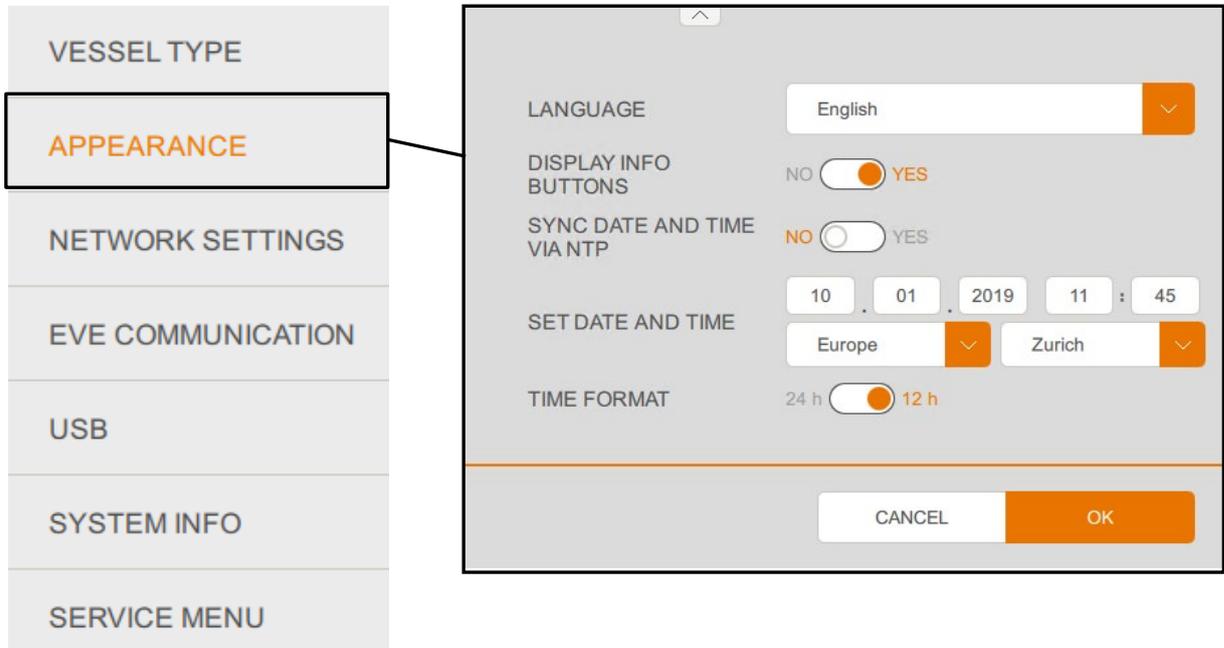


#### ATTENTION

With the selection of the culture vessel used, the permitted limit values and control settings for the corresponding vessel size are configured in the background. If the vessel size is set incorrectly, it could cause undesired behaviour from the control.

### 10.2.2 APPEARANCE – Display Settings

A variety of display settings can be made in the *APPEARANCE* menu.



#### LANGUAGE

Selection of the display language.

The desired display language can be selected using the drop-down list. The languages in the drop-down list are always displayed in English.



#### INFORMATION

Additional languages can be downloaded from the INFORS HT website to a USB stick and loaded to the device from the **USB** menu. If the desired language is not available, contact the local service partner of the device manufacturer.

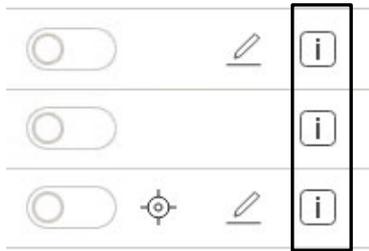
#### DISPLAY INFO BUTTONS

Switching the on-screen help off or on

This display of the info buttons for the on-screen help for the various parameters is switched on (YES) or off (NO) using the switch.



## Operation



If the display is switched on, the info buttons also appear in the main screen, in the parameter options menu display, see figure to the left illustrating an extract of the menu display.

### STIRRER

The direct function of the stirrer is to provide a **good mixing** in the bioreactor in order to achieve a **homogeneous** distribution of the culture and the added corrective agents and/or feed media. Additionally, the impellers play an indirect **role in the gassing of the bioreactor**. By shredding bigger gas bubbles into several smaller ones, the overall surface of gas bubbles inside the bioreactor is increased, leading to an **improved gas transfer**. This effect is increased with higher stirrer speeds and, consequently, the stirrer speed is often used in a **cascade for pO<sub>2</sub> control**.

OK

After pressing an info button, a dialogue appears containing basic information on the selected parameter, example to the left: parameter *STIRRER*.

### SYNC DATE AND TIME VIA NTP

If this function is activated, the touchscreen synchronizes its date and time with a network time server (NTP) present and configured in the network.



#### INFORMATION

In this case, the date and the time of the bioreactor cannot be set manually (SET DATE AND TIME).

### SET DATE AND TIME

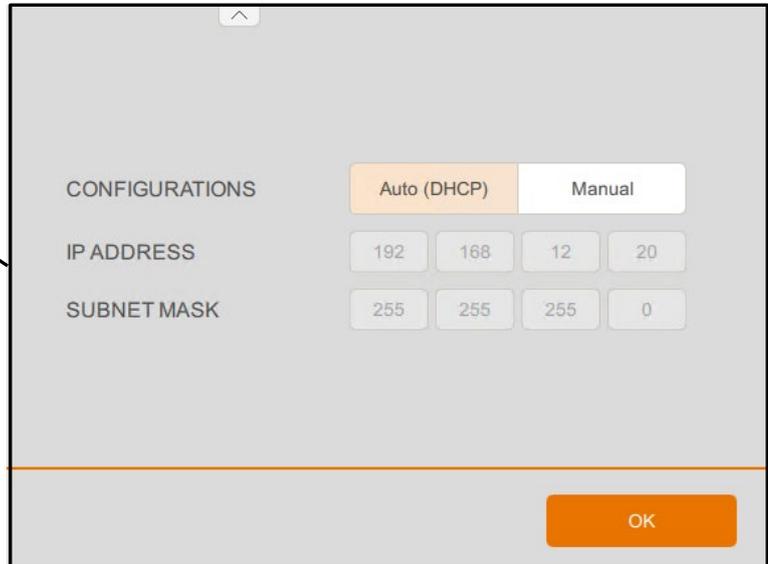
Manually enter the date and time. Can only be done provided *SYNC DATE AND TIME VIA NTP* is not switched on.

### TIME FORMAT

Switch between 12 h and 24 h time formats

### 10.2.3 NETWORK SETTINGS

The network connection of the bioreactor is configured in the *NETWORK SETTINGS* menu.



#### INFORMATION

If the bioreactor is to be integrated into an existing network, the network specifications are to be followed and the corresponding settings are to be used. Please consult your network administrator.

#### CONFIGURATIONS

Determine whether the network connection is to be automatically configured (**Auto (DHCP)**) or needs to be set up manually (**Manual**).

A DHCP server is required in the network for automatic configuration with the DHCP protocol. Please consult your network administrator.

#### IP ADDRESS

Displays the allocated IP address during automatic configuration (*Auto (DHCP)*), or can be used to enter the IP address for manual configuration (*Manual*).

#### SUBNET MASK

Displays the subnet mask address during automatic configuration (*Auto (DHCP)*), or can be used to enter the subnet mask for manual configuration (*Manual*).

**Operation**

**i** **INFORMATION**

The network connection can be used to connect the device with the bioprocess platform software eve®.

**10.2.4 E COMMUNICATION – Communication Settings**

In the eve COMMUNICATION menu, permissions for server access as well as their security settings for communication with the bioprocess platform software eve® are set.

**INFORMATION**

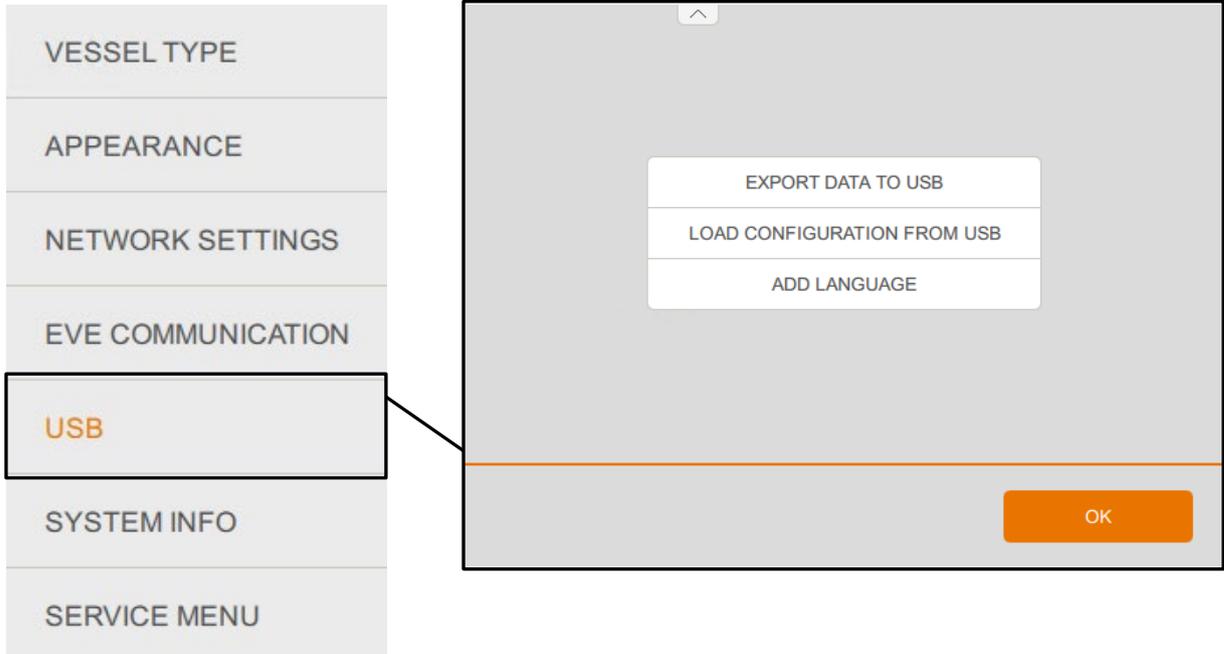
Display of the device name (*Device Name*) and its network address (*IP*, configuration under *NETWORK SETTINGS*). This information is required for configuration of the connection in eve® (device manufacturer’s bioprocess platform software)

**SERVER ACCESS**

Determine whether the bioreactor is invisible (**Hidden**), only available for read access (**Read Only**) or available for read and write access (**Read/Write**) in the OPC UA.

### 10.2.5 USB Data Export and Import from a USB Stick

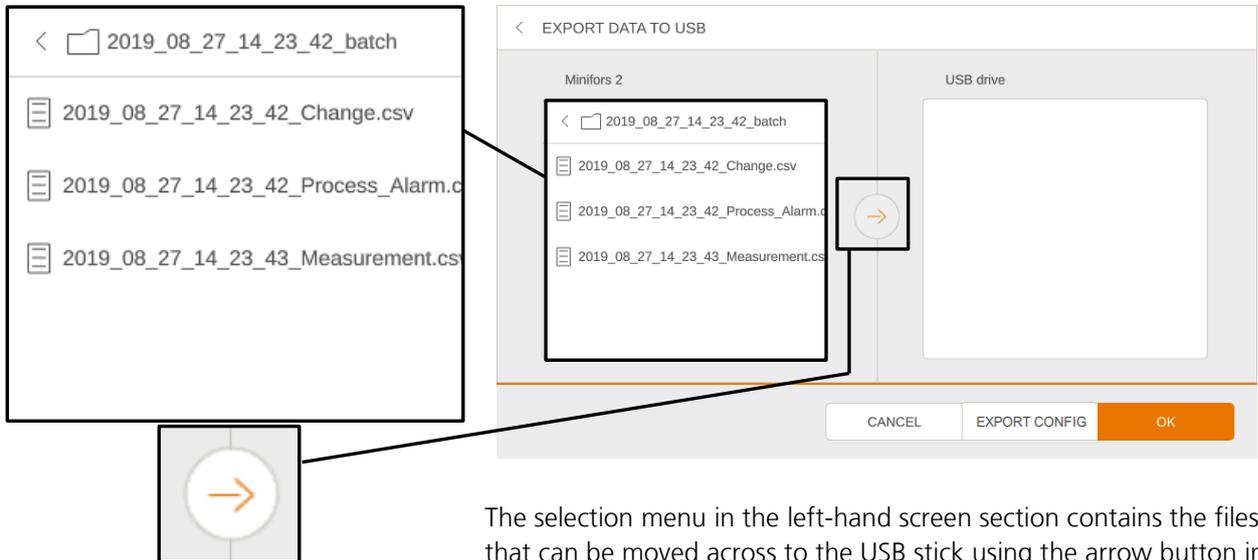
In the *USB* menu, a USB stick connected with the device's USB port can be used to import or export data.



## Operation

### EXPORT DATA TO USB

Opens the menu for data export.



The selection menu in the left-hand screen section contains the files that can be moved across to the USB stick using the arrow button in the middle.

- **EXPORT CONFIG:** to export a backup of the current device configuration as zip file to the USB stick, which can be imported again by *LOAD CONFIGURATION FROM USB*.
- **CANCEL:** to cancel and leave the menu without changes
- **OK:** to confirm data export and leave the menu.

Three files are created per batch and are ready for export. Each file name contains the start date and start time of the batch:

yyyy	=	Year
mm	=	Month
dd	=	Day
hh	=	Hours
ii	=	Minutes
ss	=	Seconds

The three files contain the following:

#### **yyyy\_mm\_dd\_hh\_ii\_ss\_Change.csv**

Log of the changes during the batch, e.g. manual input of setpoints, in CSV format. In combination with the initial state at batch start

(**EXPORT CONFIG**) the current configuration can be determined at any time.

The columns of the CSV file are:

- DateTime: Absolute date and time
- Parameter: Parameter that was changed
- Property: Property of the parameter that was changed
- NewValue: Property of the newly allocated value
- OldValue: The old value of the property

#### **yyyy\_mm\_dd\_hh\_ii\_ss\_Measurement.csv**

Log of the actual values of all parameters during the batch in CSV format. The recording interval is 1 min. If higher precision is required, SCADA software, e.g. the bioprocess platform software eve<sup>®</sup>, can be connected via OPC UA and used for recording.

The columns of the CSV file are:

- DateTime: Absolute date and time
- ProcessTime: Relative time to batch start (*batch time*)
- <ParameterName>: Actual value of the corresponding parameter

#### **yyyy\_mm\_dd\_hh\_ii\_ss\_Process\_Alarm.csv**

Log of all alarms that occurred during the batch (e.g. deviations from the setpoints and actual values) and events (e.g. sampling) in CSV format.

The columns of the CSV file are:

- DateTime: Absolute date and time
- AlarmType: Type of alarm or event
- ProcessTime: Relative time to batch start (*batch time*)
- EndAlarmTime: Time at which the alarm state was resolved
- ConfirmedTime: Time at which the alarm was confirmed on the operating panel

#### **LOAD CONFIGURATION FROM USB**

Opens the menu for importing a device configuration from the USB stick (see also **EXPORT CONFIG** in **EXPORT DATA**) including setpoints, cascades and PID settings.

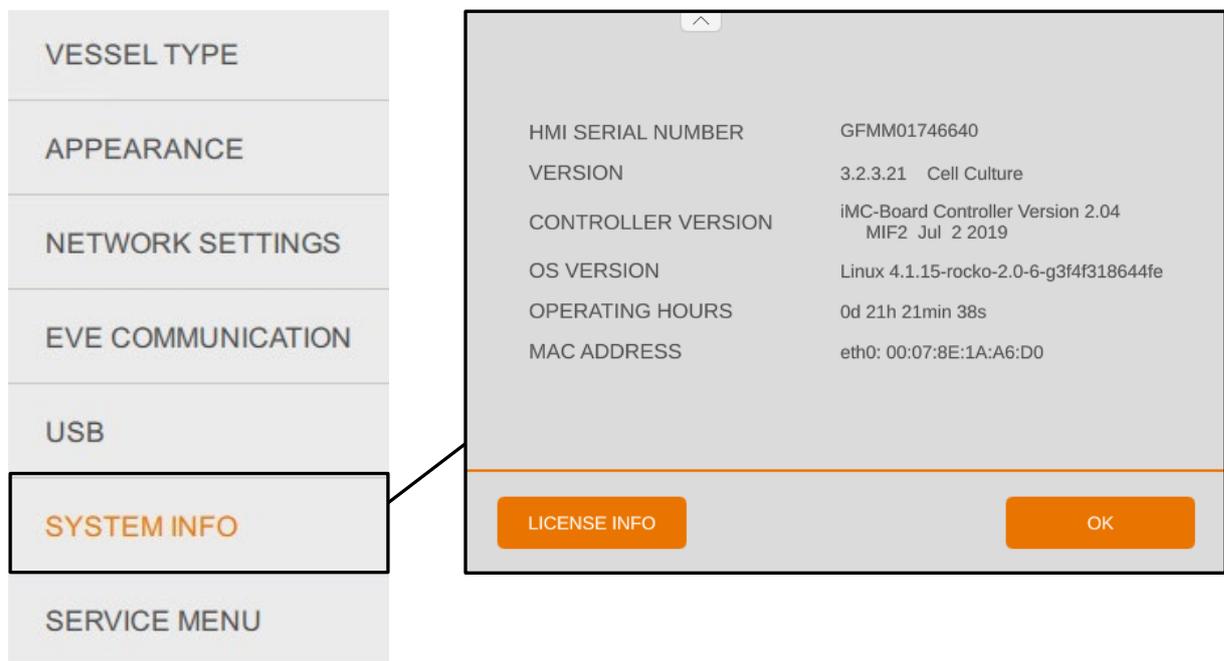
## Operation

### ADD LANGUAGE

A selection menu for data import appears. A language can be added or updated, e.g. downloaded from the INFORS HT download centre or provided by an authorised INFORS HT service partner.

### 10.2.6 SYSTEM INFO – System Information

The *SYSTEM INFO* menu displays some important system information.



- **HMI SERIAL NUMBER:** serial number of the operating panel
- **VERSION:** current firmware version & version of the device (for microorganisms or cell culture)
- **CONTROLLER VERSION:** controller version
- **OS VERSION:** version of the operating system
- **OPERATING HOURS:** operating hours of the device since start-up
- **MAC ADDRESS:** hardware address



#### LICENSE INFO

---

[AFL-2 license](#)  
[Apache-2.0 license](#)  
[Arphic-Public-License license](#)  
[Artistic-1.0 license](#)  
[BSD license](#)  
[BSD-2-Clause license](#)  
[BSD-3-Clause license](#)  
[BSL-1.0 license](#)  
[BitstreamVera license](#)  
[CC-BY-3.0 license](#)  
[CC-BY-SA-3.0 license](#)  
[Elfutils-Exception license](#)  
[FSF-Unlimited license](#)  
[FreeType license](#)  
[GFDL-1.2 license](#)  
[GFDL-1.3 license](#)  
[GPL-1.0+ license](#)  
[GPL-2.0 license](#)

Pressing **LICENSE INFO** opens a menu with the licenses of all software libraries used.

## Operation

### 10.3 Parameter - Parameter Groups

On the main screen, up to eight parameters can be simultaneously monitored and controlled. The parameters are divided into five parameter groups:

The screenshot shows the MINIFORS 2 main screen. On the left, a vertical menu lists five parameter groups: FAVORITES (highlighted in orange), MAIN, EXTENDED, EXIT GAS, and PUMPS. The main area displays a table of parameters with columns for PARAMETER, VALUE, and SETPOINT. Below the table, there is a 'Batch Time (since inoc.)' of 00:12:30 and three buttons: EDIT VIEW, SAMPLE NOW, and STOP BATCH.

PARAMETER	VALUE	SETPOINT		
Temperature	10.0 °C	37.0 °C	<input type="checkbox"/>	
Stirrer	0 min <sup>-1</sup>	55 min <sup>-1</sup>	<input type="checkbox"/>	
pH	2.00	7.00	<input type="checkbox"/>	
pO <sub>2</sub>	0.0 %	21.0 %	<input type="checkbox"/>	

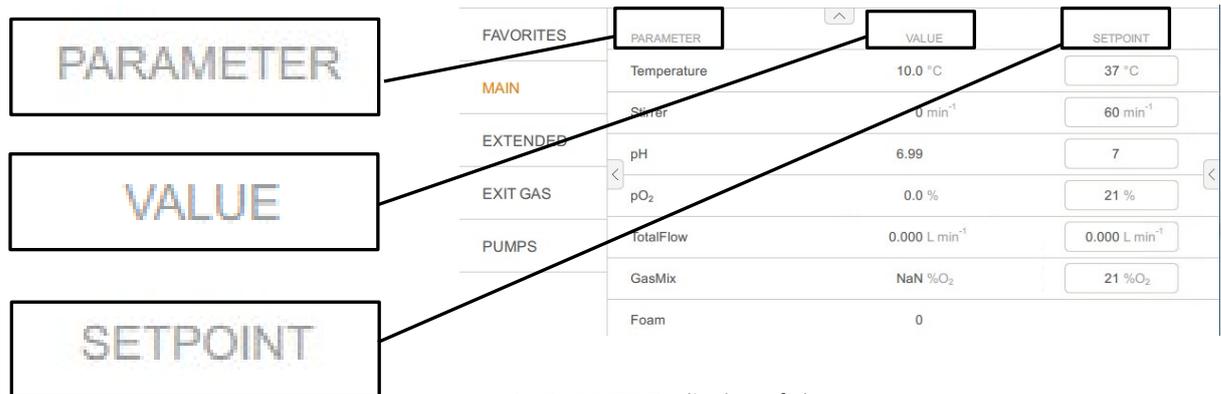
- **FAVORITES:** allows you to compile up to eight parameters from the other four parameter groups. This can be done using the **EDIT VIEW** button. For details, see the "EDIT VIEW" chapter.
- **MAIN:** contains the *Temperature*, *Stirrer*, *pH*, *pO<sub>2</sub>*, *Total Flow*, *GasMix* and *Foam* parameters.
- **EXTENDED:** contains the *Air Flow*, *Gas2 Flow*<sup>1)</sup>, *N2 Flow*<sup>2)</sup>, *O2 Flow*<sup>2)</sup>, *CO2 Flow*<sup>2)</sup>, *Air Headspace*<sup>2)</sup>, *Analog IO1* and *Analog IO2* parameters as well as the optional parameters *Balance* and *Turbidity*, if available.
- **EXIT GAS:** contains the *Exit Gas O<sub>2</sub>* and *Exit Gas CO<sub>2</sub>* parameters, provided the exit gas analysis option is available.
- **PUMPS:** contains the *Pump1* to *Pump4* parameters and also offers the *FILL* and *EMPTY* functions.

<sup>1)</sup> Version for microorganisms only

<sup>2)</sup> Version for cell culture only

### 10.3.1 Parameters – Displays and Functions

Irrespective of the selected parameter group, every parameter menu has the same three columns.



- **PARAMETER:** display of the parameter name
- **VALUE:** display of the actual value of the parameter
- **SETPOINT:** entry of the max. setpoint of the parameter

If the right-hand display is shown, further functions are available depending on the selected parameter group and parameter.

#### ON/OFF



Switches control of the selected parameter on or off

#### INFORMATION

ON/OFF is only available when a Batch is running. First start the batch with START BATCH and, if necessary, INOCULATE.

#### Calibrating

Opens the calibration menu of the selected parameter.



#### INFORMATION

The "Calibration" function is only available for the *pH*, *pO<sub>2</sub>* and *Turbidity* (variant OPTEK) parameters.

Operation is described in chapter "Calibration".

## Operation



### Editing

Opens the editor menu with the various settings for the selected parameter. Not all parameters have an editor menu.

E.g. cascades can be set here, PID settings can be adjusted, parameter alarms can be switched on or off or the pump functions can be selected.

The settings are described along with the corresponding parameter in the later parameter chapters.



### Information

Opens a dialogue containing basic information on the selected parameter.



#### INFORMATION

Display of the info button is switched on or off in the *APPEARANCE* menu for system settings.

### 10.3.2 SETPOINT - Setting the Setpoint

The setpoints can be entered in any operating state of the device for parameters that are not controlled via cascade and have a controller output. Parameter control is however only active when a batch has been started using **START BATCH** and the corresponding parameter has been activated using **ON/OFF**.

Operation

The image shows two parts of the interface. On the left, a 'SETPOINT STIRRER' dialog box is shown with an input field containing '50 min<sup>-1</sup>', a 'DELETE' button, a numeric keypad, and 'CANCEL' and 'OK' buttons. On the right, a parameter list table is shown with the 'Stirrer' parameter highlighted, and its 'SETPOINT' column also showing '50 min<sup>-1</sup>'.

PARAMETER	VALUE	SETPOINT
Temperature	10.0 °C	37.0 °C
Stirrer	0 min <sup>-1</sup>	50 min <sup>-1</sup>
pH	2.00	7.00
pO <sub>2</sub>	0.0 %	21.0 %
TotalFlow	0.000 L min <sup>-1</sup>	0.000 L min <sup>-1</sup>
GasMix	NaN %O <sub>2</sub>	21 %O <sub>2</sub>
Foam	0	

After pressing the input field in the *SETPOINT* column of the desired parameter, the key pad appears for typing in the setpoint and, if necessary, for activating the parameter (using ON/OFF)

- **OK** confirms the input, the key pad disappears.
- **CANCEL** makes the key pad disappear without making any changes.

If an inadmissible setpoint is entered, an error message is generated that prompts a correct entry within the permissible parameter setpoints.

The image shows the 'SETPOINT STIRRER' dialog box with '8000 min<sup>-1</sup>' entered. A yellow error message box is overlaid on the dialog, stating: 'Please enter a Value within the valid range: 0 – 1600 min<sup>-1</sup>'. The background shows the parameter list with 'Stirrer' selected.

Example (version for microorganisms): The *Stirrer* setpoint entry is too high. Make a new entry within the permissible values of 0 to 1600 min<sup>-1</sup>.

## Operation

### 10.3.3 Parameter Alarms

If a parameter is activated and the Batch inoculated, parameter alarms are generated after a predefined waiting time if there are unexpected deviations from the actual values and setpoints. Parameter alarms are additionally signalled by the green blinking light of the LED status indicator on the basic unit.

Parameter alarms are displayed as follows:

PARAMETER	VALUE	SETPOINT
Temperature	32.2 °C	37.0 °C
Stirrer	24 min <sup>-1</sup>	24 min <sup>-1</sup>
pH	7.00	7.00
pO <sub>2</sub>	100.0 %	100.0 %
TotalFlow ← pO <sub>2</sub>	8.00 L min <sup>-1</sup>	8.00 L min <sup>-1</sup>
GasMix	NaN %O <sub>2</sub>	21 %O <sub>2</sub>
Foam	0	

Batch Time (since inoc.)  
00:03:18

EDIT VIEW    EDIT VIEW    SAMPLE NOW

- In the parameter group that contains the parameter(s) in question, a number on a red background appears. This indicates the number of existing parameter alarms.
- The parameter in question is displayed with a red bar and a current value in red.
- A red exclamation mark highlighted in white on a red background appears in the footer.

#### Parameter Alarm pH and pO<sub>2</sub>

If necessary, the triggering of parameter alarms can be suppressed for the two parameters pH and pO<sub>2</sub>. This means that the function can be switched on and off in the editor menu of the corresponding parameter.

EDIT pH

ALARM



Example to the left: Editor menu of parameter pH with function switched on.



**INFORMATION**

For all other parameters, this function is always activated ex-factory and is neither visible for nor editable by the operator.

Pressing the symbol or swiping upwards opens the *Equipment Alarm* menu. For details, see the “Alarms – Equipment Alarm Menu” chapter.

Parameter alarms are also logged in the batch log file, see the chapter “USB Data Export and Import from a USB Stick”.

**Parameter alarm limit factory settings**

Parameter	Alarm limit	
	Value	Unit
Temperature	2	°C
Stirrer <sup>1)</sup>	50	min <sup>-1</sup>
Stirrer <sup>2)</sup>	15	min <sup>-1</sup>
pH	0.5	pH
pO <sub>2</sub>	10	%
Total Flow <sup>1)</sup>	0.3	L min <sup>-1</sup>
Total Flow <sup>2)</sup>	10	mL min <sup>-1</sup>
GasMix	10	%
Air Flow	0.3	L min <sup>-1</sup>
Gas2 Flow <sup>1)</sup>	0.3	L min <sup>-1</sup>
N <sub>2</sub> Flow <sup>2)</sup>	10	mL min <sup>-1</sup>
O <sub>2</sub> Flow <sup>2)</sup>	10	mL min <sup>-1</sup>
CO <sub>2</sub> Flow <sup>2)</sup>	10	mL min <sup>-1</sup>
Air Headspace <sup>2)</sup>	10	mL min <sup>-1</sup>

<sup>1)</sup> Version for microorganisms

<sup>2)</sup> Version for cell culture

## Operation

### 10.3.4 Cascades

Cascades can be configured for some parameters. A cascade can be used to assign a parameter to another parameter as an actuator.

**Example:**

For control of the  $pO_2$  by changing the *Gasmix* parameter, a cascade to the *Gasmix* parameter is configured for the  $pO_2$ . If the  $pO_2$  actual value is below the prescribed setpoint, the *Gasmix* is increased by the controller until the desired setpoint is reached for the  $pO_2$ .

The cascades can be configured using the editor menu of the parameter. The procedure is described in the parameter description for the parameters for which this is possible.

Parameters that are used in a cascade are identified in the main menu with an arrow and the name of the controlling parameter, and manual setpoint entry is deactivated.

	PARAMETER	VALUE	SETPOINT
FAVORITES	Temperature	11.9 °C	37 °C
MAIN	Stirrer ← pO <sub>2</sub>	3 min <sup>-1</sup>	60 min <sup>-1</sup>
EXTENDED	pH	6.99	7
EXIT GAS	pO <sub>2</sub>	19.1 %	21 %
PUMPS	TotalFlow	0.000 L min <sup>-1</sup>	0.000 L min <sup>-1</sup>
	GasMix	NaN %O <sub>2</sub>	21 %O <sub>2</sub>
	Foam	0	

Example: *Stirrer* is used in a cascade for  $pO_2$  control. It is not possible to enter a setpoint for *Stirrer*.

## 10.4 MAIN Parameter Group

The *MAIN* parameter group contains all parameters that are available by default, as well as the two parameters *GasMix* and *TotalFlow* for flow control of the individual gasses.

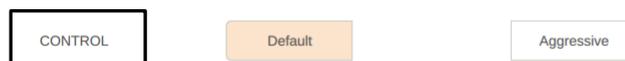
### 10.4.1 Temperature

Measures and controls the temperature in the culture vessel. The temperature controller is optimized by default for a minimum overshoot during adjustment (setting "Default"). Alternatively, the controller can be set "Aggressive" for the version for microorganisms so that temperature changes occur more quickly, but the setpoint can be exceeded for a short time during adjustment.

#### Settings (version for microorganisms only)

The controller can be switched in the editor menu of the parameter.

EDIT Temperature



The only menu item *CONTROL* contains the two options mentioned above.

### 10.4.2 Stirrer

Measures and controls the speed of the stirrer. Rotation speed depends on factors such as the size of the motor, vessel volume, culture viscosity, number and kind of impellers etc.

Stirring speed is often used in a cascade for pO<sub>2</sub> control. Cascades for pO<sub>2</sub> control can be configured in the editor menu of the pO<sub>2</sub> parameters.

### 10.4.3 pH

Measures and controls the pH in the culture vessel within a range of 2 to 12.

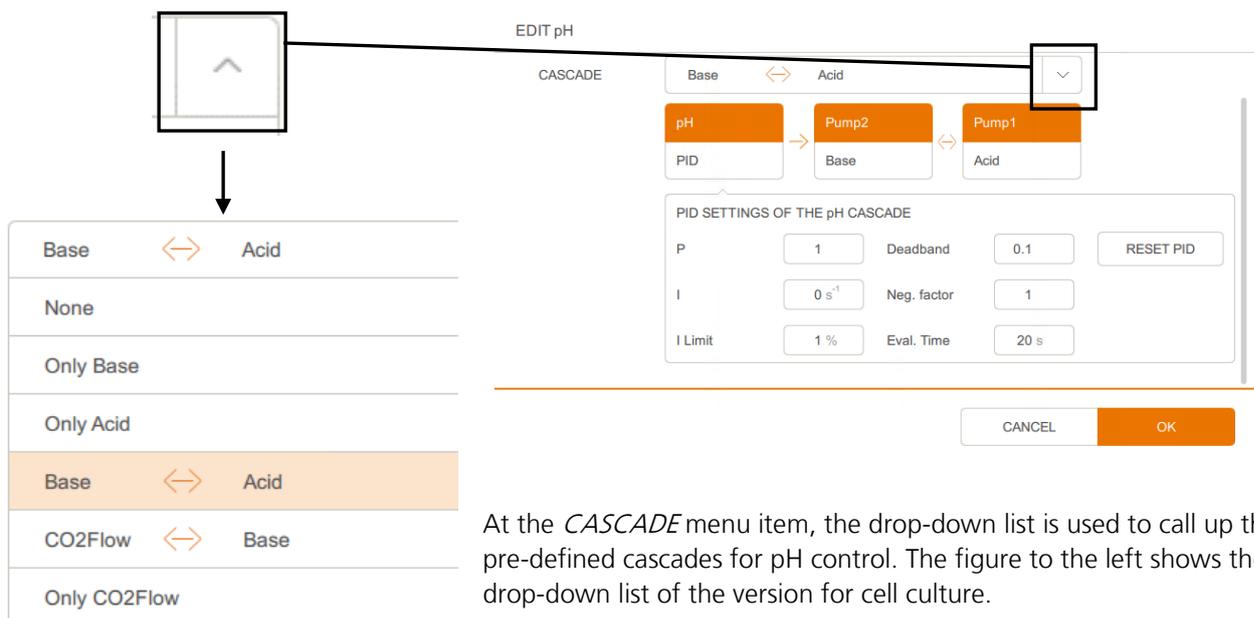
For details on the safety, technical data, usage and maintenance requirements for the pH sensors, see the separate documentation provided by the sensor manufacturer.

## Operation

The pH control can be configured using a cascade and takes place by default by adding acid and base via the two peristaltic pumps *Pump1/Acid* and *Pump2/Base*. For details on the pumps, see the “PUMPS Parameter Group” chapter.

### Settings

The settings for the cascade are made in the editor menu of the parameter.



At the *CASCADE* menu item, the drop-down list is used to call up the pre-defined cascades for pH control. The figure to the left shows the drop-down list of the version for cell culture.

The following settings are available for selection:

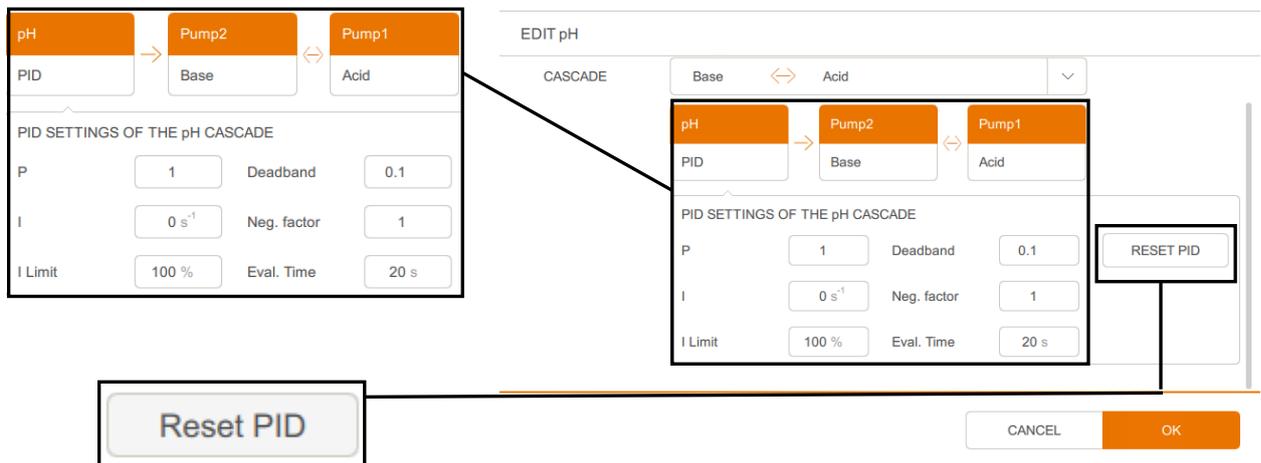
- **None:** no control, pH is only measured.
- **Only Base:** pH control takes place by adding base from *Pump2*.
- **Only Acid:** pH control takes place by adding acid from *Pump1*.
- **Base – Acid:** default setting, pH control takes place by adding base and acid.

Additional choice for version for cell cultures:

- **CO<sub>2</sub> Flow – Base:** pH control takes place by adding base and CO<sub>2</sub> (instead of liquid acid). Depending on the configuration, CO<sub>2</sub> enters via sparger or head space.
- **Only CO<sub>2</sub> Flow:** pH control only takes place by adding CO<sub>2</sub> (instead of liquid acid).

The selected setting is represented visually. In the below example, the standard setting with control via the acid and base pumps is depicted.

The PID menu is activated.



The PID settings can be adjusted here as required or, if necessary, can be reset to factory settings using **RESET PID**.

For details on the PID controller, see the “PID Controller - Basics” chapter and associated sub-chapters.

After setting the desired cascade, entries are confirmed using **OK**.

### 10.4.4 pO<sub>2</sub>

Measures the dissolved oxygen in the culture. Unlike measurements such as pH, which are calibrated to absolute measurement values, the oxygen measurement is always calibrated to a relative reference point. For this purpose, the calibration is set to 100 % relative oxygen saturation, usually with air at max. stirring speed and maximum gas flow rate.

The absolute concentration of dissolved oxygen in mmol L<sup>-1</sup> may therefore vary at 100 % saturation, depending on the process.

For details on the safety, technical data, usage and maintenance requirements for the pO<sub>2</sub> sensors, see the separate documentation provided by the sensor manufacturer.

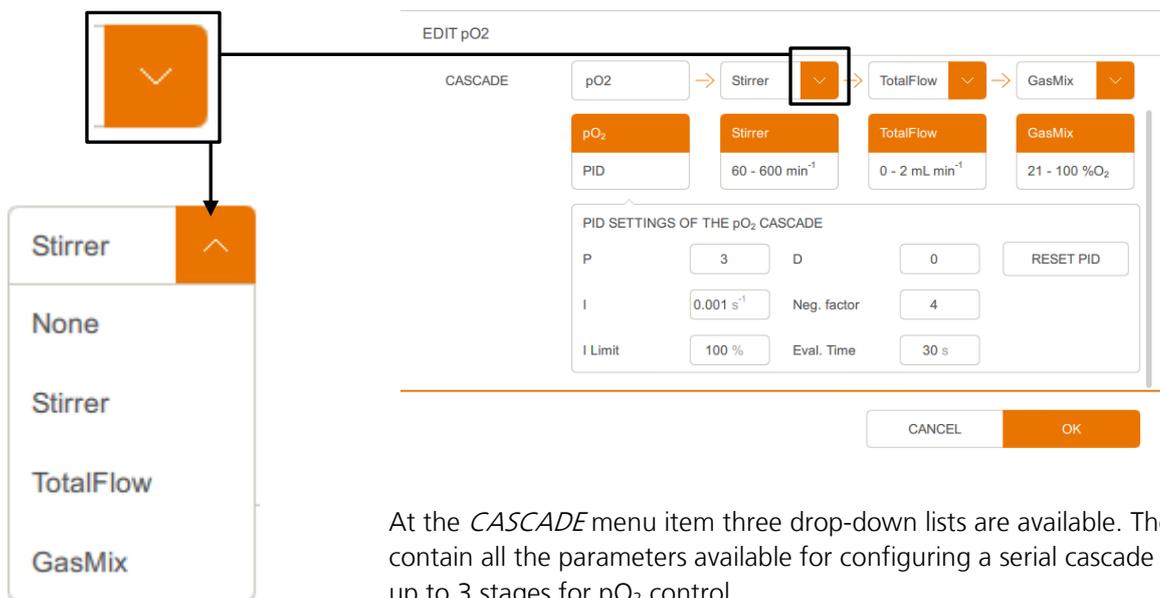
Since the pO<sub>2</sub> value cannot be directly influenced by the bioreactor, actuators must be assigned to the PID controller of the pO<sub>2</sub>

## Operation

parameter. This takes place using cascades with other parameters, such as *Stirrer* (stirrer speed), *TotalFlow* (gas flow) or *GasMix* (gas mixture).

### Settings

The settings for the cascade are made in the editor menu of the parameter.



At the *CASCADE* menu item three drop-down lists are available. They contain all the parameters available for configuring a serial cascade of up to 3 stages for pO<sub>2</sub> control.

The following settings are available for selection:

- **None:** no control, pO<sub>2</sub> is only measured.
- **Stirrer:** pO<sub>2</sub> is controlled using *Stirrer*
- **TotalFlow:** pO<sub>2</sub> is controlled using *TotalFlow*
- **GasMix:** pO<sub>2</sub> is controlled using *GasMix*.



### INFORMATION

GasMix is only available if more than one gas is used and this is set accordingly in the editor menu of the GasMix parameter.

### Serial cascades

- **Stirrer – Total Flow:** pO<sub>2</sub> is first controlled by *Stirrer* and, after reaching its maximum, it is controlled by *TotalFlow*.
- **Stirrer – Gasmix:** pO<sub>2</sub> is first controlled by *Stirrer* and, after reaching its maximum, it is controlled by *Gasmix*.

- **Stirrer – Total Flow – GasMix:** pO<sub>2</sub> is first controlled by *Stirrer* and, after reaching its maximum, it is controlled by *TotalFlow* and, after reaching its maximum, it is controlled by *Gasmix*.



**INFORMATION**

Changing the cascade(s) and restricting/expanding the ranges requires setting/checking the PID values.

The selected setting is represented visually. In the below example, the setting with control using *Stirrer* (stirrer speed) is depicted.

The PID menu is activated.

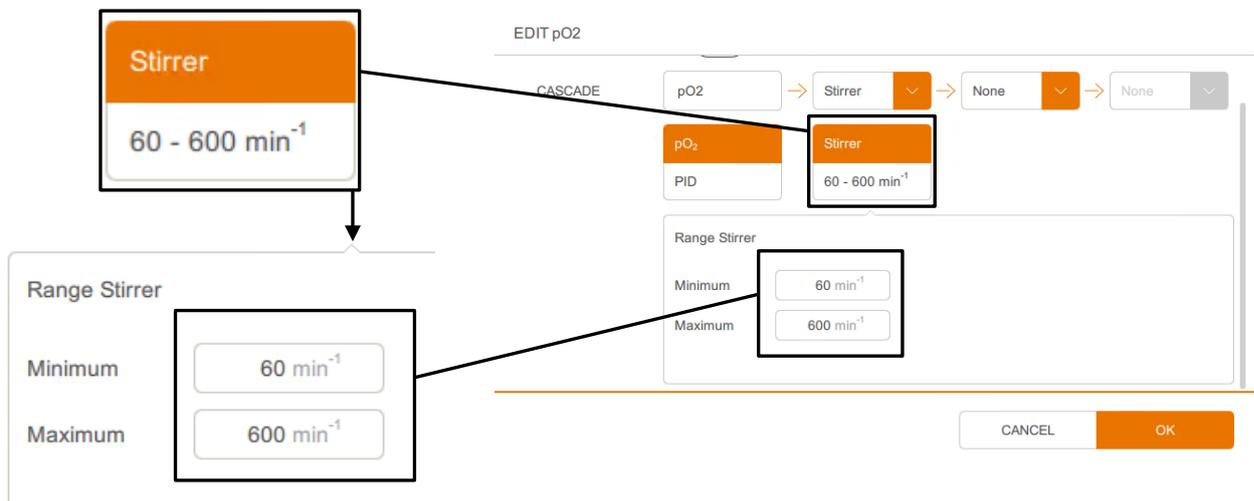


The PID settings can be adjusted here as required or, if necessary, can be reset to factory settings using **RESET PID**. For details on the PID controller, see the chapter “PID Controller - Basics” and associated subchapters.

If necessary, the value ranges used for the cascaded parameter(s) can be adjusted here.

## Operation

In the example below, the cascaded parameter *Stirrer* is selected for this purpose in the visual representation, and the input fields for *Minimum* and *Maximum* become visible.



After pressing an input field, the key pad appears for typing in the value (also see the "SETPOINT - Setting the setpoint" chapter).

After setting the desired cascade, entries are confirmed using **OK**.

### 10.4.5 Total Flow

Measures and controls the sum of the volume flows of air (*Air Flow*) and one or two connected gas(es):

- Version for microorganisms: gassing with a second gas (oxygen OR nitrogen) possible, parameter *Gas2 Flow*.
- Version for cell culture: gassing with two gases (oxygen AND nitrogen) possible, parameters *O<sub>2</sub> Flow* and *N<sub>2</sub> Flow*.

The mixing ratio of air with one or two connected gas(es) is controlled by the *GasMix* parameter. The controller calculates the setpoints for *Air Flow* and the additional flow parameter(s) on the basis of the setpoints for *Total Flow* and *Gasmix*. This allows, for example, the volume flows to be kept constant in the event of a changed gas composition, or the gas composition to be kept constant in the event of a changing volume flow. The measurement value is displayed in L min<sup>-1</sup> (version for microorganisms) or in mL min<sup>-1</sup> (version for cell culture).

The sum of the volume flows, *Total Flow*, is often used in a cascade for pO<sub>2</sub> control. Cascades for pO<sub>2</sub> control can be configured in the editor menu of the pO<sub>2</sub> parameter.

### 10.4.6 GasMix

Controls the oxygen concentration in the inlet air. This is done by mixing air and oxygen (O<sub>2</sub>) or air and nitrogen (N<sub>2</sub>). For the cell culture version, the 3-gas mixing system of air, nitrogen and oxygen is also available.

#### Settings

The configuration is made in the editor menu of the parameter, see next figure, example of cell culture version.



The only menu item available here, *FEATURE*, has the following options:

- **Only Air:** air is exclusively used, with no addition of a second gas. The gas mixture always contains 21 % oxygen. The *Total Flow* corresponds to *Air Flow*. Parameter *GasMix* is not available for use in the pO<sub>2</sub> cascade.
- **Air/O<sub>2</sub>:** the setpoint can be varied between 21 % (only air) and 100 % (only O<sub>2</sub>). *Total Flow* therefore remains constant, the ratio of *Air Flow* and *Gas2 Flow*<sup>1)</sup> or *O<sub>2</sub> Flow*<sup>2)</sup> is adjusted automatically on the basis of the setpoint of *GasMix*.
- **Air/N<sub>2</sub>:** the setpoint can be varied between 0 % (only N<sub>2</sub>) and 21 % (only air). *Total Flow* therefore remains constant, the ratio of *Air Flow* and *Gas2 Flow*<sup>1)</sup> or *N<sub>2</sub> Flow*<sup>2)</sup> is adjusted automatically on the basis of the setpoint of *GasMix*.

<sup>1)</sup> Version for microorganisms

<sup>2)</sup> Version for cell culture

#### Version for cell culture only

- **Air/N<sub>2</sub>/O<sub>2</sub>:** the setpoint can be varied between 0 % (only N<sub>2</sub>), 21 % (only air) and 100 % (only O<sub>2</sub>). *Total Flow* therefore remains constant, the ratio of *Air Flow* and *O<sub>2</sub> Flow* and *N<sub>2</sub> Flow* is adjusted automatically on the basis of the setpoint of *GasMix*.

## Operation



### INFORMATION

The oxygen content of air is 20.95 %. The device works with the rounded value 21 % for easier display.



### INFORMATION

The 3-gas mixing system always requires air and cannot be used to mix nitrogen and oxygen. Set *GasMix* to *Only Air* and control *N<sub>2</sub> Flow* and *O<sub>2</sub> Flow* individually in the parameter group EXTENDED.

After selecting the desired option, entry is confirmed using **OK**.

The gas composition *GasMix* is often used in a cascade for pO<sub>2</sub> control. Cascades for pO<sub>2</sub> control can be configured in the editor menu of the pO<sub>2</sub> parameters.

### 10.4.7 Foam

In the standard setting, measures foam formation (*Antifoam* function) and controls the addition of antifoaming agent from Pump3. The digital antifoam pump is activated as soon as the antifoam sensor comes into contact with foam.

Alternatively, the antifoam sensor can be configured as a level sensor so that *Pump3* pumps medium/liquid into the culture vessel until the desired fill level has been reached, respectively the sensor detects liquid.



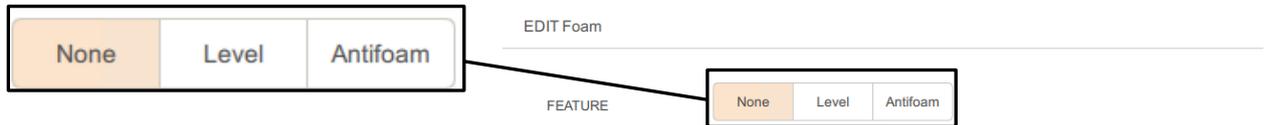
### INFORMATION

If the filling level in the culture vessel is to be kept constant by removing culture medium by Pump3 as soon as the sensor detects liquid, this can be done via the Antifoam feature and connecting the pump hoses the other way round, because direction of rotation of the pump cannot be changed.

It is important to reconnect the pump hoses in the usual way again, when changing to the "normal" function mode of the Antifoam feature!

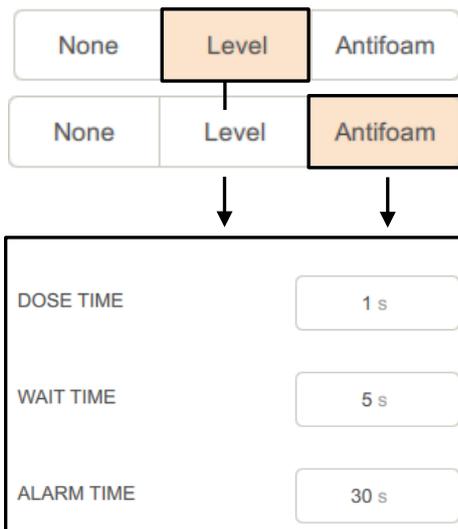
**Settings**

Selection of the foam sensor functions, as well as other possible settings, takes place in the editor menu of the parameter.



The *FEATURE* menu item has the following three options:

- **None:** no control, foam/liquid is only detected.
- **Level:** addition of culture medium (filling of the culture vessel) until sensor detects liquid.
- **Antifoam:** addition of antifoam agent as soon as sensor detects foam.



If the *Level* or *Antifoam* function is selected, additional parameter settings are possible:

- **DOSE TIME:** duration (in seconds) of the addition of antifoaming agent, respectively culture medium by *Pump3*.
- **WAIT TIME:**
  - Feature *Antifoam*: duration (in seconds) after the addition of antifoaming agent to reduce foam before more antifoaming agent is added.
  - Feature *Level*: no waiting time is needed here, duration can be set to 0 (zero).
- **ALARM TIME:**
  - Feature *Antifoam*: time (in seconds) after which a parameter alarm is triggered if foam is still detected despite the addition of antifoam agent.
  - Feature *Level*: time must be set to 0 (zero).

After pressing an input field, the key pad appears for typing in the value (also refer to chapter "SETPOINT - Setting the Setpoint".) All entries are confirmed with **OK**.

## Operation

### 10.5 EXTENDED Parameter Group

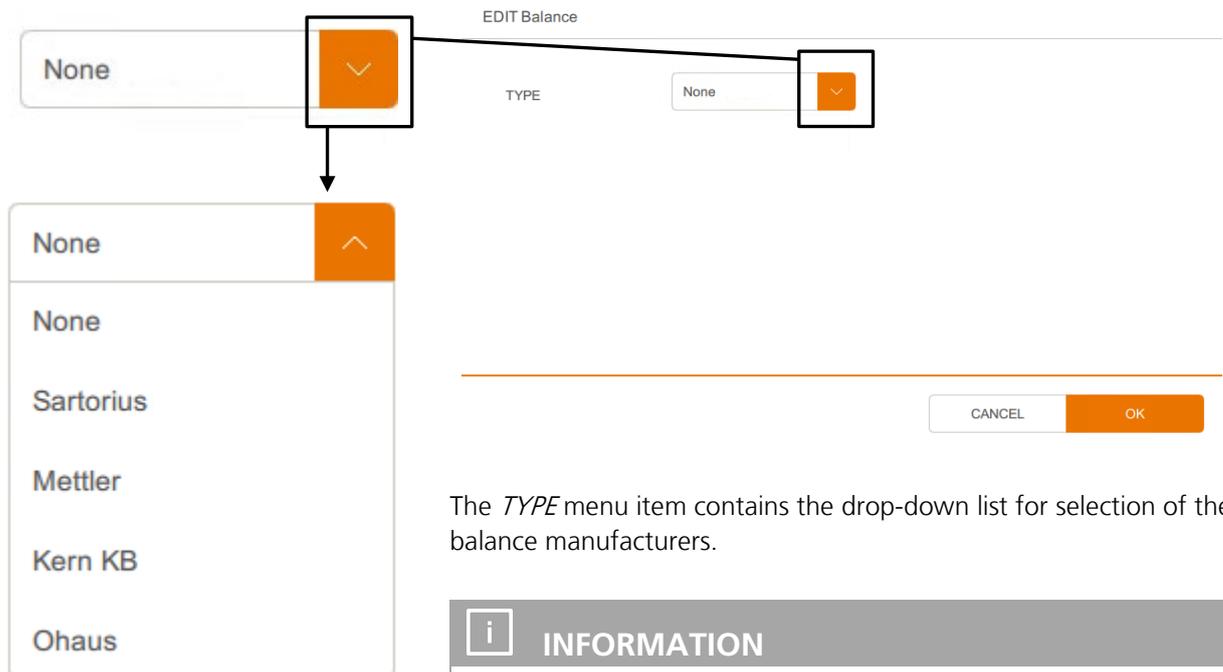
The *EXTENDED* parameter group contains all existing (Gas)Flow parameters, two parameters for analogue Inputs/Outputs and the optional parameters for weight measurement (*Balance*) and turbidity (*Turbidity*), if the respective option is connected.

#### 10.5.1 Balance (Optional)

Measures a weight, e.g. a bottle with feed solution. Can be coupled with *Pump4* (feed) to carry out gravimetric feeding. See the "*Pump4* (feed solution)" chapter for details.

##### Settings

The balance type can be configured in the editor menu of the parameter.



The *TYPE* menu item contains the drop-down list for selection of the balance manufacturers.

##### **i** INFORMATION

Balances must be configured with the following values: Baudrate 9600, 8 bits, no parity, 2 stop bits.

For a list of compatible balances or help with the connection, please contact your local INFORS HT service partner.

## 10.5.2 Air Flow, Gas2 Flow, O<sub>2</sub> Flow, N<sub>2</sub> Flow, Air Headspace, CO<sub>2</sub> Flow

All flow parameters measure and control the volume flow of the corresponding gas into the culture vessel via a mass flow controller (thermal mass meter with control valve). The measuring system is completely electronic and the measured value is displayed in L min<sup>-1</sup> (version for microorganisms) or mL min<sup>-1</sup> (version for cell culture).

Depending on the device version, the following flow parameters are present by default:

- Version for microorganisms: *Air Flow* (air) und *Gas2 Flow* (for oxygen OR nitrogen).
- Version for cell culture: *Air Flow* (air), *O<sub>2</sub> Flow* (oxygen), *N<sub>2</sub> Flow* (nitrogen), *Air Headspace* (air headspace) and *CO<sub>2</sub> Flow* (carbon dioxide).

The maximum gassing rate is determined by the vessel size used in the *VESSEL TYPE* menu. For values see main chapter "Technical Data", chapter "Specifications", "Gassing".

### Air Flow

Regardless of the existing device version and configuration of the gassing system, the setpoint for the air volume flow is ALWAYS set in the *TotalFlow* parameter. A setpoint can NEVER be set in the *Air Flow* parameter, as the oxygen concentration is ALWAYS controlled by the *GasMix* parameter, even if only air is used. For details about Total Flow and GasMix, see the corresponding chapters in chapter "Parameter Group MAIN".

### O<sub>2</sub> Flow / N<sub>2</sub> Flow

Depending on which configuration is selected in the *GasMix* parameter, setpoints for the volume flow of oxygen and/or nitrogen can be set individually.

### Air Headspace

The setpoint settings of the air volume flow for headspace gassing with air is independent of the parameters *GasMix* and *TotalFlow*.

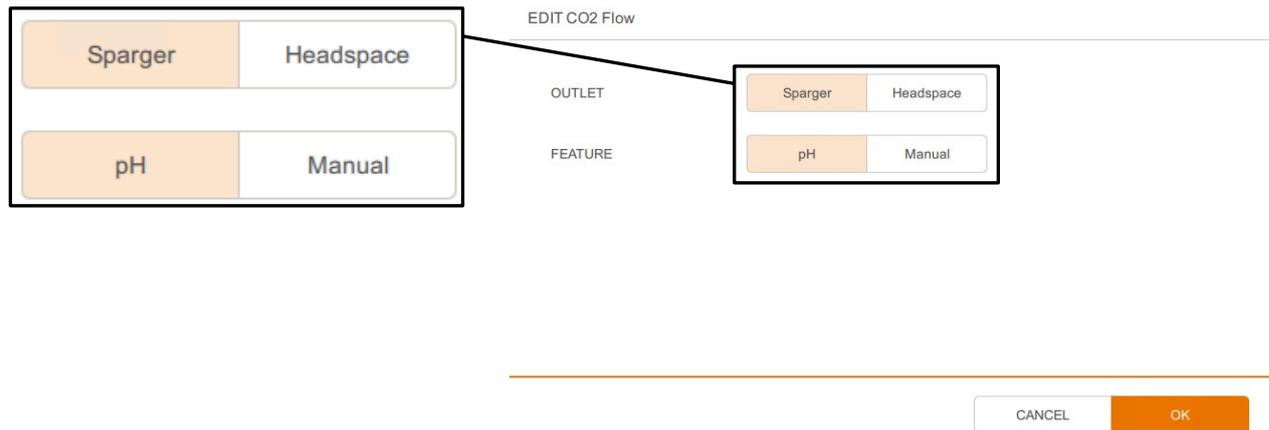
### CO<sub>2</sub> Flow

CO<sub>2</sub> can be used via parameter *CO<sub>2</sub> Flow* instead of liquid acid via the acid pump for pH control. Addition of CO<sub>2</sub> is either possible via

## Operation

sparger or headspace. The *CO<sub>2</sub> Flow* parameter can also be used separately from the pH control. In both cases, however, it is independent of the *GasMix* and *Total Flow* parameters.

The settings are made in the editor menu of the parameter.



The two menu items *OUTLET* and *FEATURE* have the following options:

- **Sparger / Headspace:** to select gas entry via sparger or headspace. Sparger gassing is set ex-factory.
- **pH / Manual:** to use CO<sub>2</sub> either for pH control (*pH*) or as individual gas flow parameter (*Manual*).

If the parameter is configured for pH control, it is automatically accepted in the pH parameter as an actuator in a cascade. In this case, the setpoint value can no longer be edited in the parameter. If it is used as a normal gassing parameter, the setpoint can be set as usual.

### 10.5.3 Turbidity (Optional)

Measures the turbidity in the culture. Depending on the installed measuring system, within a range from 0 to 4 CU (variant OPTEK) or from 0 to 1000 (variant Aquila). For more details, refer to main chapter "Options", chapter "Turbidity Measurement".

### 10.5.4 Analog IO1 & Analog IO2

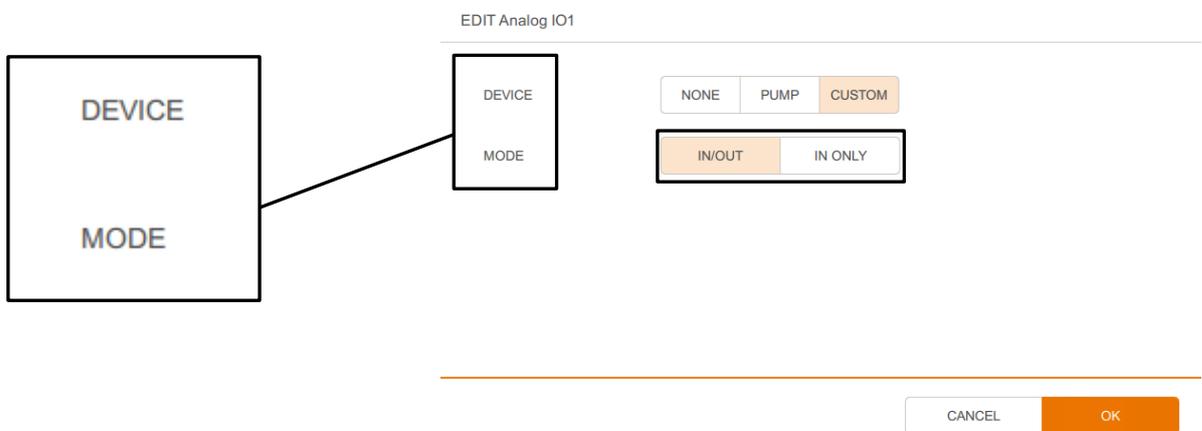
These two parameters represent two analogue 4 – 20 mA inputs/outputs and are available for the connection of two external devices. Both parameters are calibrated and scaled to a range of 0 to 100 %.

**i** INFORMATION

If external values are to be converted, this must be done via eve®, the platform software for bioprocesses.

**Settings**

In the editor menu can be set, whether the external device displays measured values only, e.g. a sensor, or whether setpoints can also be entered, e.g. for a pump.



The two menu items *DEVICE* and *MODE* offer the following options:

**DEVICE**



Setting the type of parameter, choice between *NONE*, *PUMP* or *CUSTOM* (customized).

**i** INFORMATION

Settings here are only relevant, if working with eve®, the platform software for bioprocesses.

**MODE**



Mode selection, choice between *IN/OUT* (with setpoint entry and display of current value, e.g. pump) or *IN ONLY* (measured only, display of current value, e.g. sensor).



The picture to the left shows the parameter *Analog I/O* set to *IN/OUT* mode.

## Operation

### 10.6 EXIT GAS Parameter Group

The *EXIT GAS* parameter group contains the parameters for the optional exit gas analysis. For details refer to main chapter "Options", chapter "Exit Gas Analysis".

Details on technical data, usage and maintenance requirements for the gas sensors can be found in separate documentation provided by the manufacturer.

#### 10.6.1 Exit Gas O<sub>2</sub>

Measures the oxygen concentration in the exit gas of the bioreactor using a combined gas sensor of the type BlueInOne Ferm, BlueInOne Cell or BlueVary.

The following two measuring ranges are possible, depending on the present sensor type:

- 1,0 to 50 Vol. % O<sub>2</sub> <sup>1)</sup>
- OR:
- 0 to 100 Vol. % O<sub>2</sub> <sup>2)</sup>

<sup>1)</sup> *only suitable for aerobic bioprocesses (sensor type: BlueInOne Ferm and BlueVary)*

<sup>2)</sup> *suitable for aerobic and anaerobic bioprocesses (sensor type: BlueInOne Cell and BlueVary).*

#### 10.6.2 Exit Gas CO<sub>2</sub>

Measures the carbon dioxide concentration in the exit gas of the bioreactor using a combined gas sensor of the type BlueInOne Ferm, BlueInOne Cell or BlueVary.

The following two measuring ranges are possible, depending on the present sensor type:

- 0 to 10 Vol. % CO<sub>2</sub>
- OR
- 0 to 25 Vol. % CO<sub>2</sub>

### 10.7 PUMPS Parameter Group - General Information

In the *PUMPS* parameter group, the delivery rate of the pumps can be set or monitored and the function mode of the pumps can be configured.

The screenshot shows a control interface for pumps. On the left, there is a vertical stack of four 'FILL' buttons and four 'EMPTY' buttons, with an 'OPEN AUTO FILL/EMPTY' button at the bottom. On the right, a table displays pump parameters:

PARAMETER	VALUE	SETPOINT
Pump1	0 ~ml	0 %
Pump2 ← pH	0.0 ~ml	0.0 %
Pump3	0.0 ~ml	0.0 %
Pump4	0.0 ~ml	0.0 %

Below the table, there is another vertical stack of four 'FILL' buttons and four 'EMPTY' buttons, with an 'OPEN AUTO FILL/EMPTY' button at the bottom. A line connects the 'OPEN AUTO FILL/EMPTY' button in the left panel to the one in the right panel.

In addition, the pump hoses can be manually filled or emptied by pressing and holding **FILL** or **EMPTY**.

By pressing **AUTO FILL/EMPTY**, the submenu opens with the option to set a time control for the filling and emptying of every pump.

For details on automatic filling/emptying, see the later chapter "AUTO FILL/EMPTY – Automatically Filling/Emptying Pump Tubes".

Depending on the function mode, the pumps run in analogue (continuous) operation with variable speed, or digital operation with fixed speed.

#### Example

- Analogue: 50 % = half speed = half delivery capacity
- Digital: 50 % = 100 % speed, but only active 50 % of the time = half delivery rate.

Ex works, the pumps are configured as follows:

- **Pump1:** *Acid* (addition of acid, digital), controlled by the *pH* parameter.
- **Pump2:** *Base* (addition of base, digital), controlled by the *pH* parameter.
- **Pump3:** *Antifoam* (addition of antifoaming agent, digital), controlled by the *Foam* parameter.
- **Pump4:** *Feed*: (addition of feed solution, analogue), controlled by the user.

## Operation

The figure below shows the pumps with the factory configuration.

PARAMETER	VALUE	SETPOINT	FILL	EMPTY
Pump1	0 ~ml	0 %	FILL	EMPTY
Pump2 ← pH	0.0 ~ml	0.0 %	FILL	EMPTY
Pump3	0.0 ~ml	0.0 %	FILL	EMPTY
Pump4	0.0 ~ml	0.0 %	FILL	EMPTY

OPEN AUTO FILL/EMPTY

In digital operation, pumping is used as an actuator for other parameters such as *pH* or *Foam* and receive their setpoint from the corresponding controller. This means that it is not possible to enter a setpoint.

When pumping in analogue operation (example to the left, *Pump4*), setpoints can be specified in % of pump capacity.

The totalled actual value of a pump is displayed, depending on the configuration, in the number of rotations or as an estimated volume in mL or for *Pump4* as a weight in grams in the VALUE column on the main screen.

### 10.7.1 Configuring the Pumps

The editor menu of every pump has four menu items for configuration. The figure below shows the editor menu of *Pump 1* as an example.

EDIT Pump1

TUBE TYPE	<input type="radio"/> ø 0.5 mm <input checked="" type="radio"/> ø 1.0 mm <input type="radio"/> ø 2.5 mm
FEATURE	<input checked="" type="radio"/> Acid <input type="radio"/> Feed
DISPLAY COUNT UNIT	<input type="radio"/> Count <input checked="" type="radio"/> ~ml
VALUE	0 ~ml <input type="button" value="RESET COUNT"/>

CANCEL OK

**TUBE TYPE**

To select the pump hose used.



The following pump hoses are available: 0.5 mm, 1.0 mm (standard) or 2.5 mm. On the basis of the selected hose diameter, the pumped volume can be estimated and used for the display of the totalled actual value (selection under *DISPLAY COUNT UNIT*).

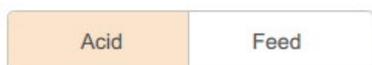


**INFORMATION**

An incorrectly set hose diameter results in an incorrectly totalled actual value.

**FEATURE**

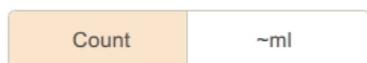
To configure the pump function operating mode.



Since the four pumps have different functions, they are described in the following chapters.

**DISPLAY COUNT UNIT**

To configure the display of the totalled actual value.



Either *Count* (number of rotations of the pump head) or *~ml* (the pumped volume estimated on the basis of the hose diameter selected under *TUBE TYPE*) can be selected.



**INFORMATION**

If a balance (*Balance*) is connected and linked with *Pump4, g* (measured pumped weight) is also available at *Pump4*.

**VALUE**

Displays the totalled actual value and to reset the counter.

0 ~ml

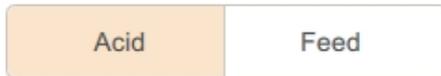


The totalled actual value of the pump is displayed here and can be reset to 0 by pressing **RESET COUNT**.

## Operation

### 10.7.2 Pump1 - Acid or Additional Feed Solution

*Pump1* can be configured for the *Acid* function (factory setting) or *Feed*.



- **Acid:** digital operating mode, is used in pH control to add acid.
- **Feed:** analogue (continuous) operating mode, can be used for addition of another feed solution, for example.

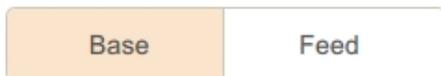


#### INFORMATION

The function of *Pump1* can also be changed by making corresponding entries in the editor menu of the pH parameter.

### 10.7.3 Pump2 - Base or Additional Feed Solution

*Pump2* can be configured for the *Base* function (factory setting) or *Feed*.



- **Base:** digital operation mode, is used in pH control to add base.
- **Feed:** analogue (continuous) operation mode, can be used for addition of another feed solution, for example.



#### INFORMATION

The function of *Pump2* can also be changed by making corresponding entries in the editor menu of the pH parameter.

### 10.7.4 Pump3 - Antifoam, Level or Additional Feed Solution

*Pump3* can be configured for the *AntiFoam* function (factory setting), *Level* or *Feed*.



- **AntiFoam:** digital operating mode, is controlled by the foam sensor (*Foam*) and used to add antifoaming agent.
- **Level:** digital operating mode, is controlled by the foam sensor (*Foam*), which is used as a level sensor, and is used to fill culture medium into the vessel.



#### INFORMATION

If the filling level in the culture vessel is to be kept constant by removing culture medium by Pump3 as soon as the antifoam sensor detects liquid, this can be done with the Antifoam function if the hoses are connected the other way round.

Please note, that if changed to “normal” Antifoam function again, hoses must be reconnected in the usual way!

- **Feed:** analogue (continuous) operating mode, can be used for addition of another feed solution, for example.



#### INFORMATION

The function of *Pump3* can also be changed by making corresponding entries in the editor menu of the *Foam* parameter.

## Operation

### 10.7.5 Pump4 - Feed Solution

Pump4 can be configured for the *Feed* function (factory setting) or, provided an optional balance is connected and the *Balance* parameter is available, it can be configured for *Balance Feed* or *Dose*.

PARAMETER	VALUE	SETPOINT
Pump1	0 ~ml	0 %
Pump2 ← pH	0.0 ~ml	0.0 %
Pump3	0.0 ~ml	0.0 %
Pump4	0.0 g	100.0 %

- **Feed:** analogue (continuous) operating mode, is used for the addition of feed solution.

The setpoint value is entered in % of pump capacity.

PARAMETER	VALUE	SETPOINT
Pump1	0 ~ml	0 %
Pump2 ← pH	0.0 ~ml	0.0 %
Pump3	0.0 ~ml	0.0 %
Pump4	0.0 g	100.0 g/h

- **Balance Feed:** analogue (continuous) operating mode, is used for the addition of feed solution. The delivery rate is controlled on the basis of the signal of the balance on which the bottle with feed solution is positioned (*Balance* parameter) to guarantee precise dosing.

The setpoint value is entered in g/h.

Feed	Balance Feed	Dose
PARAMETER	VALUE	SETPOINT
Pump1	0 ~ml	0 %
Pump2 ← pH	0.0 ~ml	0.0 %
Pump3	0.0 ~ml	0.0 %
Pump4	0.0 g	START DOSE

- **Dose:** analogue (continuous) operating mode, is used for the addition of a defined weight of feed solution.

The desired feed rate in grams is entered by pressing **START DOSE**. The key pad appears for typing in the desired dosing weight.

ENTER A DOSE WEIGHT

80 g

DELETE X

1	2	3
4	5	6
7	8	9
ABC	0	.

CANCEL OK

PARAMETER	VALUE	SETPOINT
Pump1	0 ~ml	0 %
Pump2 ← pH	0.0 ~ml	0.0 %
Pump3	0.0 ~ml	0.0 %
Pump4	0.0 g	STOP DOSE

As soon as the dosing process starts, **STOP DOSE** is available. By pressing **STOP DOSE**, the dosing process can be stopped at any time, and by pressing **START DOSE** again, the dosing process can be resumed in the same way.

After the defined amount of feed solution has been added, a new dosing process can be started with a new setpoint value.

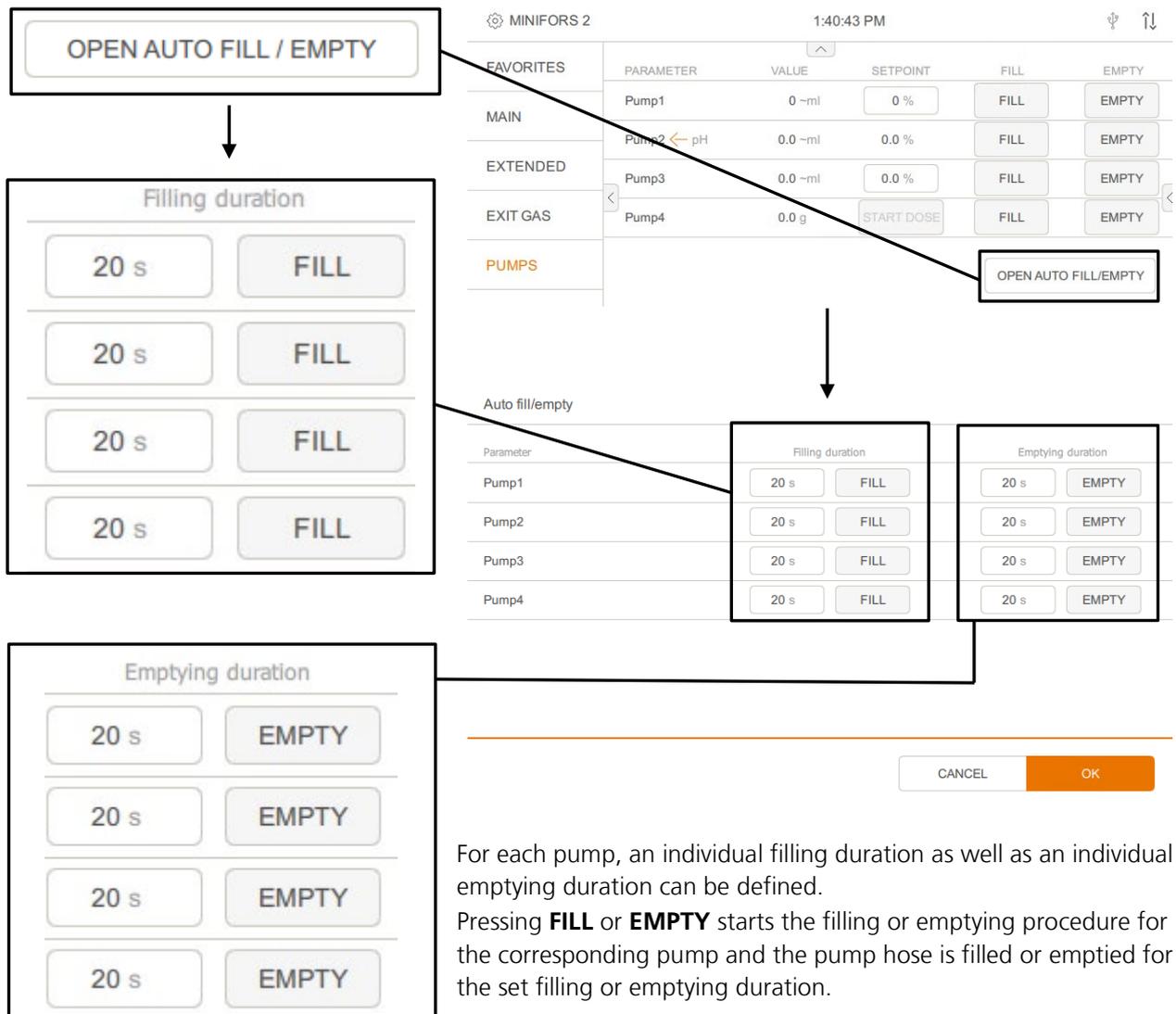
**i** INFORMATION

For the *Balance Feed* and *Dose* functions, the additional input fields for adjusting the parameters of the PID controller are available in the editor menu of *Pump4*. For details on the PID controller, see the chapter "PID Controller - Basics" and associated chapters.

**Operation**

**10.7.6 AUTO FILL/EMPTY – Automatically Filling/Emptying Pump Hoses**

Pressing **OPEN AUTO FILL/EMPTY** in the pump menu, opens the submenu for automatic filling and emptying of the pump hoses.



For each pump, an individual filling duration as well as an individual emptying duration can be defined. Pressing **FILL** or **EMPTY** starts the filling or emptying procedure for the corresponding pump and the pump hose is filled or emptied for the set filling or emptying duration.

Filling duration	
5 s	STOP
13 s	STOP
20 s	FILL
10 s	STOP

If a filling or emptying procedure is active, the remaining filling or emptying time is displayed. The filling or emptying process can be stopped at any time by pressing **STOP**. Pressing **FILL** or **EMPTY** again will restart the process.

The menu cannot be closed while at least one filling or emptying procedure is active. The menu can be closed using **OK** as soon as all filling or emptying procedures are completed.

## 10.8 Calibration

Sensors for measurement of pH, pO<sub>2</sub> and turbidity (variant OPTEK only) are usually recalibrated before each cultivation. Depending on the sensor and measurement system, either a 2-point calibration or a 1-point calibration or a zero adjustment is sufficient. Detailed information on calibration can be found in the separate documentation provided by the sensor manufacturers.

### 10.8.1 Calibrating the pH Sensor - General Information

The calibration must be carried out before sterilisation, i.e. before installing the pH sensor in the culture vessel.



#### INFORMATION

If the pH sensor has already been calibrated externally, the bioreactor will use this data and the calibration procedure on the operating panel is not necessary.

Depending on the variant selected, the device is configured for pH measurement with the digital pH sensors of the type InPro 3253i ISM from the manufacturer METTLER or the type Easyferm Plus ARC from the manufacturer HAMILTON. The pH buffers and their temperature dependencies are stored in these pH sensors and are automatically detected during calibration. It is therefore not necessary to carry out a separate temperature measurement of the buffer solution.

## Operation

Detailed information on calibration, general use, service and maintenance can be found in the separate documentation provided by the sensor manufacturers.

### 10.8.2 Calibrating the pH Sensor - Procedure

Proceed as follows to calibrate the pH sensor at the operating panel:

Procedure

1. Connect the sensor cable. For details, see the main chapter "Before Cultivation", chapter "Connecting the pH Sensor".
2. Carefully remove the cap with storage solution from the pH sensor and rinse the sensor with distilled water. Do not rub it.



#### ATTENTION

Wiping or rubbing the pH sensor after rinsing can generate an electrostatic charge. This can greatly increase the response time and generate incorrect measured values. At most, lightly dab the pH sensor after rinsing, **NEVER** wipe or rub.

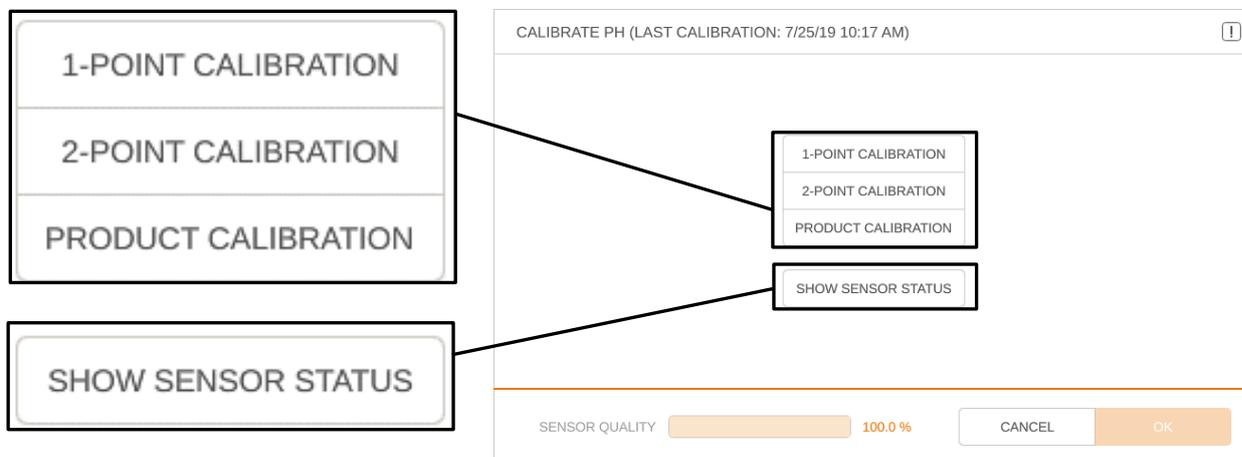


#### INFORMATION

Only sensor type Easyferm Plus ARC: the ERROR Glass resistance too high which may appear after initialization can be ignored. It may occur if the sensor is in contact with air or non-conductive liquid such as distilled water.

3. Call up the calibration menu of the pH parameter.

The menu display changes after a short initialization phase.

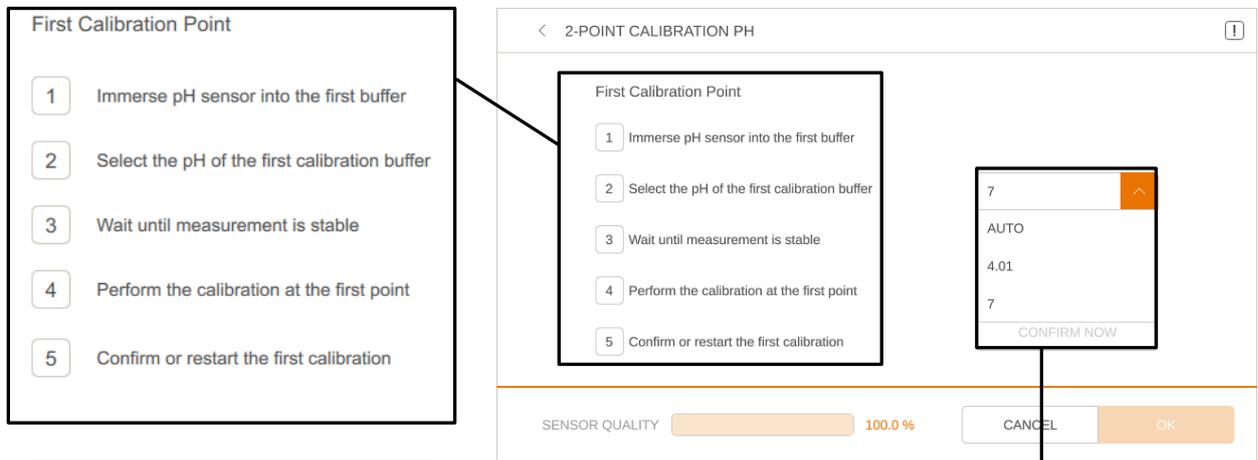


- Header: Date and time of the last calibration
- **1-POINT CALIBRATION:** to select 1-point calibration
- **2-POINT CALIBRATION:** to select 2-point calibration
- **PRODUCT CALIBRATION:** to select product calibration (for details, see the “pH product calibration” chapter).
- **SHOW SENSOR STATUS:** shows data and values produced by the firmware of the sensor manufacturer that is integrated in the sensor. For more details see section “Sensor Status”.
- HAMILTON sensors only: shows sensor quality within a range of 0 to 100 % in the footer.

4. Select 2-point calibration.

## Operation

The menu display changes to the first calibration point and shows the following:



- Left-hand side: guides step-by-step (1 – 5) through the calibration of the first reference value.
- Right-hand side:
  - Drop-down list for selection of the reference value. If the connected sensor allows the use of different calibration buffers or an automatic detection of the calibration buffer ("AUTO"), it can be selected. Otherwise, the calibration buffer to be used is displayed.
  - Measured value display
  - **CALIBRATE POINT**: to start calibration for 1<sup>st</sup> reference.
  - **CONFIRM NOW**: to confirm calibration and continue with 2<sup>nd</sup> reference.

5. Hold the pH sensor into the appropriate buffer solution of the first calibration point (step 1).
6. If possible, select the reference value or automatic buffer recognition (step 2).

The current pH measured value appears, **CALIBRATE POINT 1** is activated, i.e. the button turns orange.

7. Wait until the measured value is stable (step 3).

6.89



8. Press **CALIBRATE POINT 1** to start calibration (step 4).

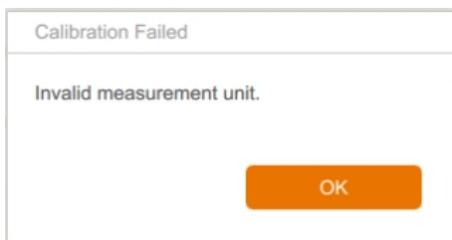


**CONFIRM NOW** slowly turns orange and indicates the ideal waiting time until a stable measured value is reached

**i** **INFORMATION**

If the measured value is assumed to be already stable, the waiting time can be skipped by pressing **CONFIRM NOW** to continue with the second calibration point.

9. Press **CONFIRM NOW** (step 5).  
The calibration point is stored.



**i** **INFORMATION**

If the calibration process fails, an error message is displayed with a corresponding message. Restart the calibration in this case.

If the calibration is successful, the menu display changes automatically to calibrate the second calibration point. The step-by-step guide (steps 6 - 10) through the calibration remains the same as for the first point (steps 1 - 5). After rinsing the pH sensor with distilled water, the same ERROR may occur. This can also be ignored here.

After successfully storing the 2nd calibration point via **CONFIRM NOW**, the calibration is completed and the menu can be exited via **OK**.

**Sensor Status**

SHOW SENSOR STATUS is used to call up data and values that are output by the firmware of the sensor manufacturer integrated in the sensor. In addition to sensor type and calibration information, the following two values are displayed for METTLER ISM sensors:

- ACT (Adaptive Calibration Timer in days): determines the time of the next calibration to ensure optimum measurement performance. It is reset to its initial value after successful calibration.
- DLI (Dynamic Lifetime Indicator in days): displays the number of days remaining and is preset by the sensor manufacturer.

## Operation

### 10.8.3 pH Sensor Product Calibration

It is possible to adjust the calibration curve to the current process conditions using product calibration. This could be necessary if there is a possibility of drift of the displayed pH during a long-term cultivation, for example.



#### INFORMATION

Product calibration can only be carried out and is only effective if the externally measured and entered pH value does not deviate from the original pH value by more than 2 pH units.

Proceed as follows for a product calibration:

Procedure

1. Call up the calibration menu of the pH parameter and wait for the short initialisation phase to complete.



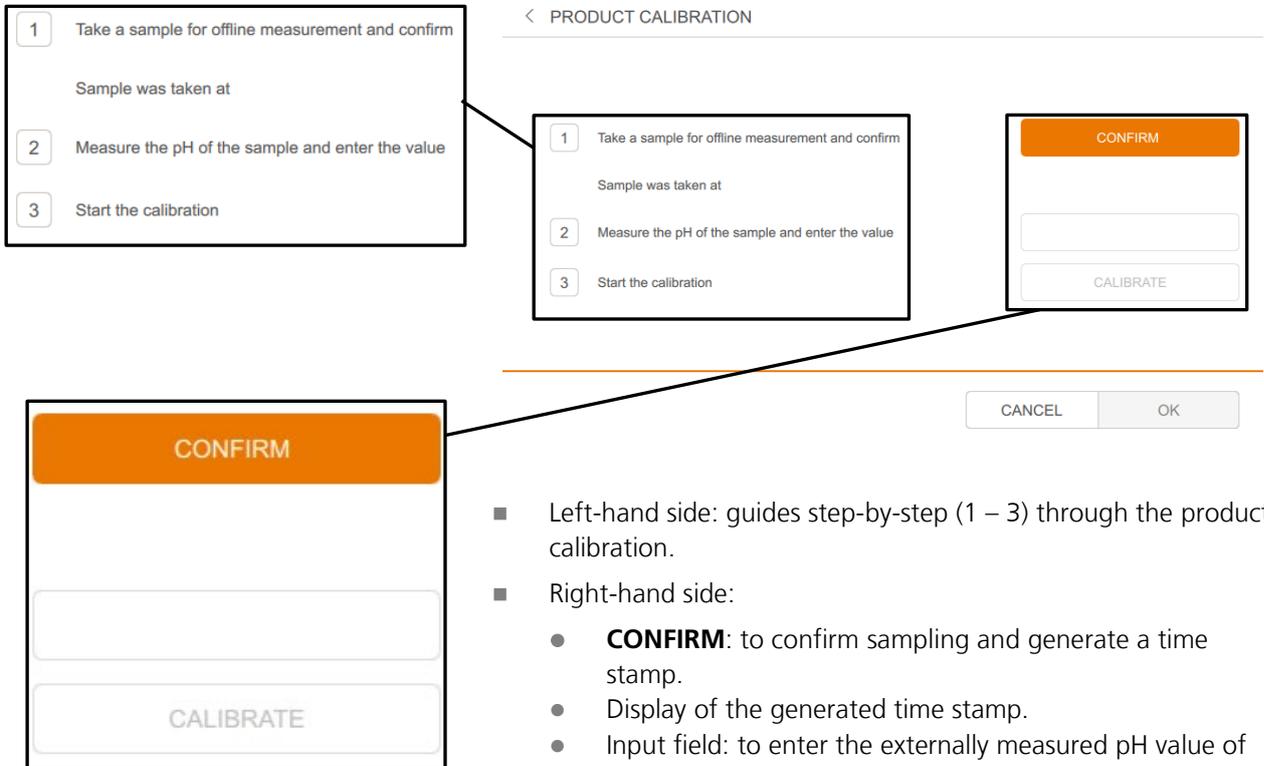
#### INFORMATION

The various menu displays of the initial calibration menu are not displayed in detail in this chapter, they are shown in "Calibrating the pH Sensor - Procedure".

**PRODUCT CALIBRATION**

2. In the menu display that follows the initialisation phase, press **PRODUCT CALIBRATION**

The menu display changes and now displays the following:



- Left-hand side: guides step-by-step (1 – 3) through the product calibration.
- Right-hand side:
  - **CONFIRM**: to confirm sampling and generate a time stamp.
  - Display of the generated time stamp.
  - Input field: to enter the externally measured pH value of the sample.
  - **CALIBRATE**: to start product calibration.

3. Take a sample from the process (in the culture vessel).

There are two possible approaches:

- a) Confirm the sampling (generate a time stamp), carry out a laboratory measurement of the pH value for the sample, enter the measured value and carry out product calibration.
- OR:
- b) Confirm the sampling (generate a time stamp), leave the calibration menu and carry out the product calibration with an external measured value at a later time.

## Operation

Procedure

CONFIRM

8/21/19 11:26 AM

6.9

CALIBRATE

PRODUCT CALIBRATION  
ACTIVE

### Variant a)

1. Press **CONFIRM**.

The date and time of the sampling are now displayed.

2. Carry out a laboratory measurement of the pH value for the sample.
3. Enter the measured pH value of the sample, in the example to the left, pH 6.9.
4. Press **CALIBRATE** to start calibration.

5. Wait until the calibration is complete.
6. Confirm the calibration with **OK** and leave the menu.

In the calibration menu, Active is displayed under PRODUCT CALIBRATION to show that a product calibration was carried out and is active.

Date and time are displayed in the header of the menu.



### INFORMATION

A new 2-point or 1-point calibration cancels the product calibration.

Procedure

### Variant b)

1. Press **CONFIRM**.

The date and time of the sampling are now displayed.

2. Leave the calibration menu using **OK** and carry out a laboratory measurement of the pH value for the sample at a later time of your choosing.

PRODUCT CALIBRATION  
Sample Taken

In the calibration menu, Sample Taken is displayed under PRODUCT CALIBRATION to show that sampling was carried out but product calibration is not yet active.

If a sample is lost, step 1 can be repeated.

3. To carry out product calibration, proceed as in Variant a) from step 3.

#### 10.8.4 Calibrating the pO<sub>2</sub> Sensor - General Information

A 1-point calibration to 100 % is usually sufficient for exact measurement and should be carried out before each cultivation. If required, a 2-point calibration to 100 % and 0 % is also possible.



##### INFORMATION

The prerequisites for exact calibration results can be found in the separate documentation of the sensor manufacturer. The calibration conditions and how they are achieved are defined by the operator and are not the subject of this operating manual.

Depending on the variant selected, the device is configured for pO<sub>2</sub> measurement with the digital pO<sub>2</sub> sensors of the type InPro 6860i ISM from the manufacturer METTLER or the type Visiferm DO ARC from the manufacturer HAMILTON



##### INFORMATION

pO<sub>2</sub> sensors are preconfigured by the device manufacturer to the measured value %-sat. Replacement sensors must also be configured by the device manufacturer.

#### 10.8.5 Calibrating the pO<sub>2</sub> sensor - Procedure

The following example describes a 2-point calibration of a pO<sub>2</sub> sensor in the medium after autoclaving. For the 100 % calibration, gassing with air is used, for the 0 % calibration, nitrogen is used.

For this, both gases must be connected and ready for operation, unused gases must be switched off.

## Operation



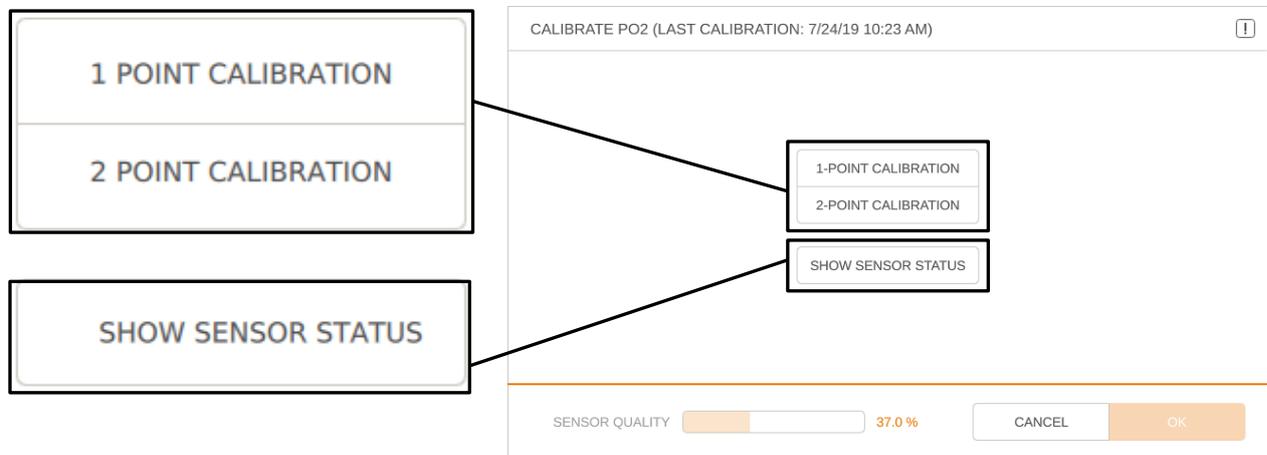
### INFORMATION

If necessary, enter setpoints for temperature and pH, activate parameters and press **START BATCH** and wait until the desired operating temperature and the expected pH have been reached.

Proceed as follows:

Procedure

1. Call up the calibration menu of the pO<sub>2</sub> parameter.  
The menu display changes after a short initialization phase:



- Header: Date and time of the last calibration
- **1 POINT CALIBRATION**: to select 1-point calibration.
- **2 POINT CALIBRATION**: to select 2-point calibration.
- **SHOW SENSOR STATUS**: shows data and values produced by the firmware of the sensor manufacturer that is integrated in the sensor. For more details see section "Sensor Status" of chapter „Calibrating the pH Sensor – Procedure“.
- HAMILTON sensors only: shows sensor quality within a range of 0 to 100 % in the footer.

2. Select 2-point calibration.

The menu display changes to the first calibration point and shows the following:

**First Calibration Point**

- 1 Select the value of the first calibration point
- 2 Optionally set setpoints for 100% pO<sub>2</sub>
- 3 Evaluate the sensor data
- 4 Perform the calibration at the first point
- 5 Confirm measure or restart first calibration

- Left-hand side: guides step-by-step (1 – 5) through the calibration of the first reference value.
- Right-hand side:
  - Drop-down list for selection of the reference value. If the connected sensor allows the use of different reference values or an automatic detection of the reference value (“AUTO”), it can be selected. Otherwise, the calibration buffer to be used is displayed.
  - **SET TO xx %**: setpoint setting to activate gassing and the stirrer for calibration in the medium.
  - Measured value display
  - **CALIBRATE POINT**: to start calibration for 1<sup>st</sup> reference.
  - **CONFIRM NOW**: to confirm calibration and continue with 2<sup>nd</sup> reference.

3. If possible, select the reference value 100 % (step 1).

**CALIBRATE POINT 1** is activated, i.e. the button turns orange.

**i** INFORMATION

This enables a calibration of the sensor outside the medium, i.e. without active gassing for a standard calibration outside the medium, which is not described here.

4. Press **SET TO 100 (%)** (step 2).

The gassing with air is activated, the stirrer is switched on at the same time.

## Operation

5. Wait until the medium is saturated with oxygen, i.e. wait until the measured value is stable (step 3).
6. Press **CALIBRATE POINT 1** to start calibration (step 4).  
Gassing and stirrer are stopped.



**CONFIRM NOW** slowly turns orange and indicates the ideal waiting time until a stable measured value is reached.

### INFORMATION

If the measured value is assumed to be already stable, the waiting time can be skipped by pressing **CONFIRM NOW** to continue with the second calibration point.

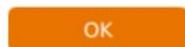
7. Press **CONFIRM NOW** (step 5).  
The calibration point is stored.

### INFORMATION

If the calibration process fails, an error message is displayed with a corresponding message. Restart the calibration in this case.

#### Calibration Failed

Phase reading during calibration is not stable.



If the calibration is successful, the menu display changes automatically to calibrate the second calibration point. The step-by-step guide (steps 6 - 10) through the calibration remains the same as for the first point (steps 1 - 5).

Proceed with the second point (0 % calibration) in the same way as for 100 %. After pressing **SET TO 0 %**, a dialogue will appear asking to check whether nitrogen is connected (version for microorganisms) or whether the nitrogen supply is turned on (version for cell cultures). If necessary, perform the corresponding step and confirm with **OK**. Then the gassing with nitrogen is activated, and the stirrer is switched on at the same time.

After successfully storing the 2nd calibration point via **CONFIRM NOW**, the calibration is completed, and the menu can be exited via **OK**.

### 10.8.6 Calibrating the Turbidity Sensor - General Information

Optek turbidity sensors (optional) are pre-calibrated in the factory. Inserts are available for reference measurement.

Due to the different light absorption of different media, zero point calibration of the turbidity sensor should be performed before each cultivation process. This can be done either **before or after** sterilisation, depending on the application in question.

#### Conditions for zero point calibration of the sensor

The sapphire windows of the optical density sensor must be clean and free of air or gas bubbles.

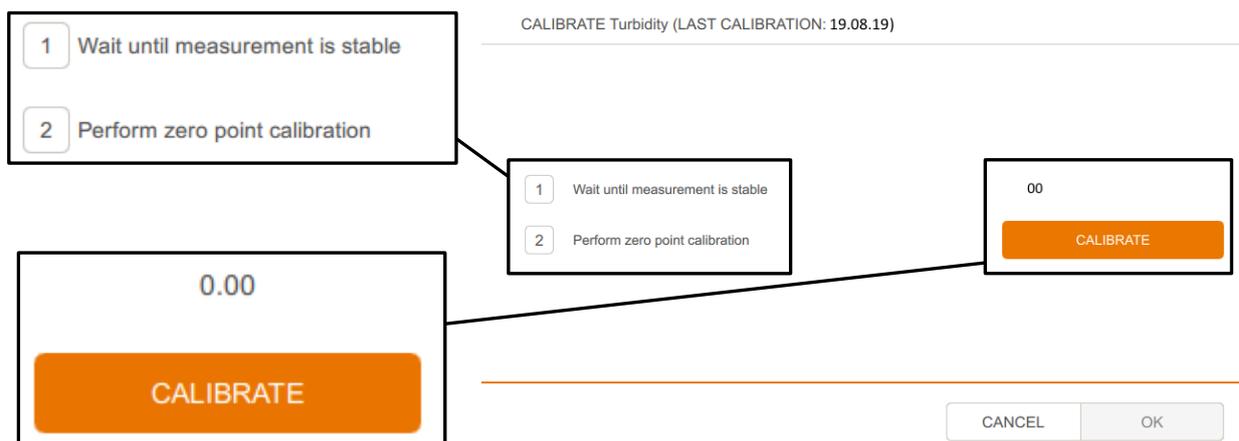
The light absorption of the medium before activation of the gassing and before inoculation can be used as a reference value for the zero point.

### 10.8.7 Calibrating the Turbidity Sensor - Procedure

To calibrate the zero point of the turbidity sensor (option, system Optek only), proceed as follows:

Procedure

1. Connect the sensor cable.
2. Call up the calibration menu of the *Turbidity* parameter.  
The menu shows:



- Header: date of the last calibration
- Left side: calibration sequence (2 steps)
- Right side: measured value and **CALIBRATE**: to calibrate the zero point.

## Operation

3. Wait until the measured value is stable.
4. Press **CALIBRATE**.

If the calibration was successful, **OK** is activated and can be pressed to confirm the calibration and leave the menu.

## 10.9 PID Controller – Basic Principle

For some parameters, PID controllers (*Proportional Integral Derivative* controller) are used.

### 10.9.1 Table with Setting Values for PID Controller

Setting value	Description
P (Prop. Term)	Proportional factor: The greater the discrepancy between the setpoint value and the actual value the greater the controller output
I (Integ. Term [1/s])	The integral factor aggregates all errors over the time. If the setpoint is not achieved using the proportional factor, the integral factor adjusts the output successively until the setpoint value is achieved. An integral factor set too high will lead to oscillation of the control loop.
D (Diff Term [s])	The differential quotient calculates the change in the actual value over the time and counteracts this change to limit any overshoot.
Neg. Factor	The negative factor can be used to add weighting to two-sided control (+100 to -100 %) (e.g. heavy acid, light alkali). In the process 1 is the balance and 0.5 or 2 equate to the half or double the controller output accordingly. Example: Nitrogen influences the pO <sub>2</sub> value less than oxygen, thus a negative factor of 2 can compensate for the reaction of the controller.
Deadband	If a dead band is entered, no control is implemented within this value at either side of the setpoint value (symmetrically, + / -). I.e. the controller output is = 0. The dead band is used for pH control.
I Limit (Integ. Limit [%])	The integral influence is used to ensure that the integral factor cannot increase over an indefinite period. This limits erroneous accumulation. The integral influence is set between 0 and 100 % of the controller output.
Eval Time [s]	The evaluation time determines the intervals in seconds at which the PID value is recalculated. The controller speed is defined this way. A scanning time of 10 seconds is a good average value.

### 10.9.2 Useful Information for Changing PID Controller Settings

To adjust the PID controller settings, proceed as follows:

Procedure

1. For readjustment of a PID controller, start with the setting for the proportional factor. Select a proportional band width as large as possible.
2. Reset the integral factor and the differential quotient to zero.

## Operation

3. Increase the proportional factor until the controller causes the actual value to oscillate.
4. Measure the oscillation duration, e.g. with the bioprocess platform software *eve*® from the manufacturer.
5. Halve the proportional factor and vary the integral factor between the reciprocal value of the doubled and quadrupled oscillation duration.

### 10.9.3 Adjusting PID Settings



#### ATTENTION

Inappropriate changes to the PID controller settings may have a negative effect on the fermentation/cultivation process and cause loss of property.

Therefore, only change factory settings of the PID controller(s), if you are fully aware of the consequences or after consulting the manufacturer!

PID control may be configured for parameters *pH*, *pO<sub>2</sub>* and *Pump4* (Function *Balanced Feed*). This is done in the editor menu of the corresponding parameter and therefore described there.

### 10.10 Alarms – Equipment Alarm Menu

There are two types of alarm that appear in the *Equipment Alarm* menu:

- **Parameter alarms:** display of deviations from actual values and setpoints for parameters after a predefined waiting time. Also refer to chapter "Parameter Alarms".
- **Equipment errors:** if equipment errors occur repeatedly or cannot be resolved, inform an authorised INFORS HT service partner. Also refer to the different interferences tables in main chapter "Interferences".

The *Equipment Alarm* menu is only available when there are open or unconfirmed alarms. Otherwise the alarm symbol (a red exclamation mark highlighted in white on a red background) is hidden in the lower screen edge.

Operation

FAVORITES	PARAMETER	VALUE	SETPOINT
MAIN	Temperature	32.2 °C	37.0 °C
	Stirrer	24 min <sup>-1</sup>	24 min <sup>-1</sup>
EXTENDED	pH	7.00	7.00
EXIT GAS	pO <sub>2</sub>	100.0 %	100.0 %
PUMPS	TotalFlow ← pO <sub>2</sub>	8.00 L min <sup>-1</sup>	8.00 L min <sup>-1</sup>
	GasMix	NaN %O <sub>2</sub>	21 %O <sub>2</sub>
	Foam	0	

Batch Time (since inoc.)  
00:03:18

Buttons: EDIT VIEW, SAMPLE NOW

Pressing the alarm symbol or swiping upwards opens the *Equipment Alarm* menu.

DESCRIPTION	STATE	CONFIRMATION
Alarm_PowerFailDuringRunningBatch	Resolved	<input type="checkbox"/>
Alarm_ControllerCommunicationFailure	Open	<input type="checkbox"/>
Temperature too high	Open	<input type="checkbox"/>
TotalFlow too high	Resolved	<input type="checkbox"/>

- **DESCRIPTION:** describes the type of alarm.
- **STATE:** status display of the alarm, open or resolved
  - Open alarms are displayed in red and with the word *Open*
  - Resolved alarms are displayed in green and with the word *Resolved*
- **CONFIRMATION:** to confirm the alarm and delete it from the list. The entry in the batch log remains.

## Cleaning and Maintenance

# 11 Cleaning and Maintenance

The following chapters describe in detail how the culture vessel and accessories and the basic unit are cleaned and, as required, stored.

In addition, the chapter contains a maintenance plan and corresponding descriptions for the procedures to be performed by the operator.

## 11.1 Cleaning Agent and Disinfectant

Intended use	Allowed products/tools
Culture vessel	Water and a non-scratch, non-abrasive sponge or washing-up brush; lab washer with special washing agent (for industry and lab use)
Cleaning agent for denaturation of proteins (e.g. exit gas cooler)	0.1 N NaOH
Cleaning agent for smaller component parts (e.g. exit gas cooler, dip tube)	Ultrasonic bath
Cleaning agent for surfaces	Water
Disinfectant for surfaces	Ethanol, 70 %
Decalcifier for the device	Amidosulfonic acid (in liquid form)

## 11.2 Cleaning the Culture Vessel - Routine Cleaning

The culture vessel and accessories can be cleaned as soon as they have cooled down after autoclaving.



### ATTENTION

Household washing-up liquid and soap (in particular cream soaps) can collect in glass pores and impair later cultivations.

Never clean culture vessels and accessories with household soap and use special cleaning agent (for industrial and lab use) in the lab washer.

The following method describes a routine cleaning between two cultivations. It takes place with the culture vessel completely assembled and the accessories completely mounted.

This does not include the sensors, with the exception of antifoam or level sensors from the device manufacturer. To avoid damaging the other sensors during the routine cleaning, they are first removed and then cleaned separately according to the third-party manufacturer guidelines and then stored, if necessary. Also see the "Removing Sensors" chapter and "Cleaning Sensors".

Proceed as follows to carry out a routine cleaning of the culture vessel:

### Procedure

1. Carefully unscrew the sensors (except antifoam/level sensors) by hand (no tools!) from the vessel top plate ports and place them to the side for separate cleaning according to the manufacturer guidelines.
2. Completely fill the culture vessel with 0.1 N NaOH.
3. Fit the top plate on the vessel and secure it.
4. Hang the culture vessel on the basic unit.
5. Couple the motor.
6. Switch on the device at the power switch.
7. At the operating panel, start the Batch (process) using **START BATCH** and stir strongly for 2 hours with the stirrer function (parameter *Stirrer*).

## Cleaning and Maintenance



### INFORMATION

It is recommended to warm the 0.1 N caustic soda to 60 °C and to prolong the duration of stirring for dealing with persistent residue of foam or protein.

8. At the operating panel, stop the Batch (process) using **INOCULATE** and **STOP BATCH**.
9. Switch off the device at the power switch.
10. Let the motor cool down.  
When the motor has cooled down:
11. Uncouple the motor.
12. Remove the top plate and carefully place it so that it does not lie on top of components(!)
13. Empty the culture vessel.
14. Thoroughly rinse the culture vessel with distilled water.

### 11.3 Removing the Vessel Top Plate and Accessories

All accessories must be removed for thorough cleaning of the individual parts of the culture vessel. This is described in the following chapters. The cleaning itself is described in the chapter "Cleaning and Storing Individual Parts".

The cleaning of the hoses with pump heads, the basic unit, operating panel and the exit gas cooler are described in separate chapters.

Sensors from third-party manufacturers are cleaned according to their manufacturer's specifications.

#### 11.3.1 Removing the Exit Gas Cooler

Proceed as follows:

Procedure

1. Unscrew the exit gas cooler from the vessel top plate port by hand.  
Ensure that the O-ring does not get lost.
2. Gently release the hose clamp with the hand wheel, pull off the exit gas filter and dispose of it.
3. Remove the pressure hose piece to thoroughly clean the exit gas cooler. (For details see the chapter "Cleaning the Exit Gas Cooler".)

## Cleaning and Maintenance

### 11.3.2 Removing the Sensors

Proceed as follows:

Procedure

#### pH, pO<sub>2</sub>

1. Carefully unscrew the sensors by hand (no tools!) from the vessel top plate ports.
2. Clean/service the sensors according to the sensor manufacturer guidelines.

Procedure

#### Antifoam/level sensor

1. Loosen and remove the fastening screw beside the sensor by hand.
2. Loosen the slotted-head screw at the clamping adapter.
3. Carefully remove the sensor from the clamping adapter.
4. Pull the clamping adapter out of the vessel top plate port by hand.

Ensure that the outer O-ring at the clamping adapter does not get lost and that the insulation is not damaged.



#### INFORMATION

The sensor can be pulled out of the vessel top plate port along with the clamping adapter. After subsequently unscrewing the slotted-head screw on the clamping adapter, the sensor can be pulled out of the clamping adapter.

### 11.3.3 Removing Hoses, Filters and Pump Heads

To later clean reagent hoses and pump heads, they must be removed from the reagent bottles and from components of the culture vessel.



#### INFORMATION

To avoid damage, never dismantle the pump heads. Always replace a damaged pump head along with the pump hose, and vice versa.

Proceed as follows:

Procedure

1. Remove cable ties (e.g. with a side cutter) so that the hoses are not damaged.

## Cleaning and Maintenance

2. Pull hoses off the culture vessel and the reagent bottles.
3. Remove and dispose of filters for pressure equalisation and hoses from reagent bottles.
4. Ensure that the inlet air filter is clean, dry and not blocked. If this is not the case, dispose of it.



### INFORMATION

If the filter for pressure equalisation and the corresponding hoses have been used several times, ensure that the filters are always dry and clean.

5. Dispose of the exit gas filter (see also chapter "Removing the Exit Gas Cooler").

### 11.3.4 Removing Blanking Plugs

Proceed as follows:

#### Blanking plugs in 10 mm vessel top plate ports

Procedure

1. Loosen and remove the fastening screw beside the blanking plugs by hand.
2. Pull the blanking plug out of the vessel top plate port by hand. Ensure that the O-ring at the blanking plug does not get lost.

#### Blanking plugs in 12 mm/Pg13.5 vessel top plate ports

Procedure

1. Loosen the blanking plug with a hexagon socket spanner and remove it by hand. Ensure that the O-ring does not get lost.

### 11.3.5 Removing the Septum Collar and Septum

Proceed as follows:

Procedure

1. Loosen the blanking plug with a hexagon socket spanner in the septum collar and remove it by hand. Ensure that the O-ring does not get lost.
2. Unscrew the septum collar out of the port by hand.
3. Remove the septum from the port and dispose of it.

## Cleaning and Maintenance

### 11.3.6 Removing Addition Port Adapters, Feed Needle and Temperature Sensor Immersion Pocket

Proceed as follows:

Procedure

1. Loosen and remove the fastening screw between the addition port adapters and/or feed needle(s) as well as beside the immersion pocket by hand.
2. Pull the addition port adapters and if necessary, the feed needle(s) from the vessel top plate ports by hand.
3. Pull the temperature sensor pocket out of the vessel top plate port.

Ensure that the O-rings on the addition port adapters and at the immersion pocket do not get lost.

### 11.3.7 Removing the Vessel Top Plate



#### ATTENTION

If the vessel top plate presses against long components they could bend because of the weight of the top plate.

Always position the vessel top plate so that it does not lie on top of components.

Proceed as follows to remove the vessel top plate:

Procedure

1. As far as possible, remove mounted parts before lifting the top plate.
2. Remove the knurled nuts on the top plate by hand (no tool!) and place them to the side.
3. Hold the glass vessel with one hand and carefully lift the top plate vertically upwards from the vessel with the other until the stirrer shaft and other long components can no longer come into contact with the glass vessel.



#### INFORMATION

If the top plate cannot be easily lifted from the glass vessel, respectively the O-ring (top plate seal), execute slight tilting movements in order to detach it from the O-ring.

4. If necessary, now remove components that have not yet been removed.

## Cleaning and Maintenance

Never remove the stirrer shaft!

5. Check the glass vessel for damage (cracks, fissures, scratches) and replace if necessary.

### 11.3.8 Removing the Sparger and the Dip Tube(s)

Straight spargers and dip tubes can be removed from the outside of the vessel top plate. Curved spargers and dip tubes can only be removed from the inside of the vessel top plate.

Since this device uses ring spargers and straight dip tubes, removal from the inside of the vessel top plate is described here. This means that the vessel top plate is already removed.

Proceed as follows:

Procedure

1. Loosen and remove the fastening screw beside the sparger/dip tube by hand.
2. Loosen the slotted-head screw at the clamping adapter.
3. Carefully pull the sparger/dip tube from the bottom out of the clamping adapter.
4. Pull the clamping adapter out of the vessel top plate port by hand.

Ensure that the outer O-ring at the clamping adapter does not get lost.

### 11.3.9 Removing the Impeller(s)

Before removing the stirrer(s), it is advisable to measure and note the position for later correct mounting.



#### INFORMATION

The mounting heights defined ex-factory for both impeller types (Rushton and pitched bladed) in all vessel sizes can be found in the main chapter "Technical Data", chapter "Specifications", chapter "Mounting heights impellers ex-factory".

For removal proceed as follows:

Procedure

1. Loosen – do not remove! - the grub screws on the impeller(s) with the Allen
2. Carefully pull the impeller off the stirrer shaft.

## Cleaning and Maintenance

### 11.4 Cleaning and Storing Individual Parts

The procedure described here applies to the following individual parts:

- Vessel
- Accessories such as blanking plugs, spargers, dip tubes, addition port adapters etc.
- Reagent bottles
- Vessel top plate, with regard to its particular characteristics
- Cold finger (optional, version for microorganisms)

#### Particulars when cleaning the top plate

- Do not place the top plate on the stirrer shaft.
- Never removed the drive hub and stirrer shaft!



#### INFORMATION

Cleaning of the sensors, hoses and pump heads as well as the basic unit and the exit gas cooler are described in separate chapters.

Proceed as follows for cleaning:

Procedure

1. Clean parts with distilled water and a soft sponge or in the dishwasher.  
Ensure that the deposits in the dip tubes and feed needles are removed. Use 0.1 N caustic soda solution followed by distilled water as necessary. For this, see chapter "Cleaning the Culture Vessel".
2. Dry all parts, including the inner parts of the dip tubes, spargers and feed needles.
3. Check all O-rings for cracks or damage. Replace them if necessary.
4. Store the vessel, vessel top plate and accessories in a clean, dry state in a location where they cannot be physically damaged (e.g. by falling), or prepare them for the next cultivation.

## Cleaning and Maintenance

### 11.5 Cleaning the Sensors

Apart from antifoam and level sensor, all sensors are cleaned and maintained according to the descriptions of the sensor manufacturer.

Procedure

1. Clean the sensors according to the sensor manufacturer guidelines.
2. Prepare the sensors for the next cultivation or, if necessary, service and/or store them according to the sensor manufacturer guidelines.

### 11.6 Cleaning the Hoses and Pump Heads

Proceed as follows to clean the reagent hoses and pump heads:

Procedure

1. Thoroughly rinse the hoses with the pump heads with water.
2. Carefully dry all hoses and, if necessary, blow out with clean-compressed air.



#### INFORMATION

To avoid damage, never dismantle the pump heads. Always replace a damaged pump head along with the pump hose, and vice versa.

### 11.7 Cleaning the Super Safe Sampler



#### ATTENTION

Risk of damage to the sampling system from unsuitable cleaning methods or cleaning agent (such as acids, bases or solvents, for example).

- Only use water or a mild soap solution for cleaning.
- The sterile filter must remain dry at all times.

Proceed as follows to clean the sampling system:

Procedure

1. Fill the culture vessel with water or a mild soap solution.  
Or: Remove the sampling hose from the dip tube and hold it in a vessel, e.g. a beaker, with water or a soap solution.

## Cleaning and Maintenance

2. Place the syringe on the automatic valve and pull out the plunger to rinse the sampling system.

When using a soap solution:

3. Then rinse the sampling system thoroughly with water.



### INFORMATION

If the test record requires that the culture is killed off after cultivation by autoclaving the culture vessel, the valves of the sampling system may become stuck due to residue of the culture solution. In such a situation, it would be better to autoclave the sampling system separately in a beaker of water (hoses filled with water, filter removed).

## 11.8 Cleaning the Exit Gas Cooler

If the exit gas cooler is only lightly soiled, an ultrasonic bath for approx. 15 minutes is sufficient to clean it.

However, if foam has entered the exit gas cooler during cultivation, it must be cleaned thoroughly.

To do so, proceed as follows:

### Procedure

1. Put the exit gas cooler into 0.1 N NaOH for 4 hours.
2. Rinse the exit gas cooler thoroughly with water.
3. Put the exit gas cooler into an ultrasonic bath for 2 to 5 minutes.
4. Flush the exit gas cooler with ethanol (70%).
5. Thoroughly rinse the exit gas cooler with distilled water.

## 11.9 Cleaning the Basic Unit and Operating Panel

Proceed as follows to clean the surface of the basic unit and the operating panel as required:

### Procedure

1. Switch off the device at the power switch.
2. Disconnect the device from the power supply.
3. Wipe all surfaces with a damp cloth.  
Clean with an appropriate disinfectant as necessary.
4. Clean the screen with a wipe suitable for computer or laptop screens.

## Cleaning and Maintenance

### 11.10 Maintenance Plan



#### **WARNING**

Non-compliance of this maintenance plan contains a high risk!

It is the responsibility of the user, that this maintenance plan is complied with. Non-compliance will lead to exclusion of liability (see General Terms and Conditions).

The required maintenance for reliable operation is described in the following chapters.

Reduce the maintenance intervals in case increased abrasion is detected during regular checks.

Contact the manufacturer for questions concerning maintenance.

## Cleaning and Maintenance

### To be carried out by operator

Interval	Maintenance work
Before each cultivation	Check all hoses and hose lines.
	Check cables for damage and kinks.
	Check that O-rings and gaskets are leak-proof, replace if necessary.
	Check the integrity of all glass parts (vessel, reagent bottles) and replace if necessary.
	Check all filters and replace if necessary. Replace the exit gas filter.
	If necessary, calibrate the sensors.
After every cultivation	Autoclave and clean the culture vessel and accessories.
As required	Clean the basic unit and operating panel.
	Decalcify the device.

### To be carried out by qualified personnel

Interval	Maintenance work
Every 6 months	Check and calibrate measurement sections (temperature, pH, etc.) with a simulator

## 11.11 Decalcifying the Device

Limescale could block installed parts, lines or valves in the basic unit. It may be necessary to decalcify the device if certain faults occur in the temperature control or gassing system.

Note the following points, before beginning the procedure:

- Be sure to respect the in chapter “Technical Data” specified inlet pressure.
- To warm up the decalcifier and pump it into the basic unit, use a chiller or a water bath and an external pump.
- During decalcification, the decalcifier flows in a circuit between the basic unit and the chiller/water bath.
- Use amidosulfonic acid in liquid form as decalcifying agent.

## Cleaning and Maintenance



### ATTENTION

Amidosulfonic acid can crystallise in case of overdosage and cause loss of property!

When preparing the decalcifying liquid, observe and follow the manufacturer's instructions for correct dosage and application!

- For the mixture, calculate 5 litres of water plus the capacity of the water bath/chiller including the hoses.

Proceed as follows for decalcifying:

#### Procedure

1. Mount the exit gas cooler into the port of the vessel top plate and connect to the basic unit.  
Ensure that the valve for the exit gas cooler water supply is open. Open it, if necessary.
2. Hang the culture vessel on the basic unit (hang the vessel holder on hooks on the thermal block).
3. Fill the chiller/water bath with the prepared decalcifying liquid.
4. Connect the chiller or water bath to the water inlet and outlet on the basic unit using hoses.
5. To open the corresponding valves in the basic unit, set the temperature on the operating panel to 4 °C (cool).
6. Set the chiller/water bath to 20 °C to 40 °C.
7. Switch on the pump at the chiller/water bath.
8. Let the decalcifier flow through the device for an hour.
9. Connect the water inlet hose on the device to tap water.
10. Hang the water outlet hose of the device at the spout.
11. Rinse the device for an hour.

## 12 Interferences

The following section describes possible reasons for interferences and how to resolve them. Reduce the service intervals in correspondence with the actual loads if interferences become increasingly common. Contact the manufacturer or licensed dealer for interferences that cannot be resolved by following the above instructions.

### 12.1 Interferences Basic Unit and Operating Panel

Interference		
Device does not work		
Possible cause	Remedy	By
Device is not switched on	Switch on the device at the power switch.	Operator
Power supply of the device is interrupted.	<ul style="list-style-type: none"> <li>■ Check if the plugs are connected</li> <li>■ Check the mains connection.</li> </ul>	Operator
Device fuse is blown.	Replace the fuse. If the fault occurs more than once, contact the INFORS HT representative.	Operator
LED flashes red, Equipment Alarm is shown on the display, power failure during a running Batch (process).	Acknowledge the alarm message. The Batch is automatically started again.	Operator
LED flashes red, Equipment Alarm is shown on the display, control system communication is interrupted.	Acknowledge the alarm message. If the alarm returns, contact the INFORS HT representative.	Operator
LED flashes red, Equipment Alarm is shown on the display, pressure in the culture vessel is too high.	Acknowledge the alarm message, if necessary replace the exit gas filter or reduce the gas flow rate.	Operator

## Interferences

### 12.2 Interferences Drive System

Interference		
Motor does not start.		
Possible cause	Remedy	By
Motor not properly connected.	Switch off the device. Check cable connections and connect correctly as necessary.	Operator
The <i>Stirrer</i> parameter is not activated.	Activate the <i>Stirrer</i> parameter.	Operator
<i>Stirrer</i> parameter setpoint = 0.	Set <i>Stirrer</i> parameter setpoint > 0.	Operator
The $pO_2$ parameter is activated and set to oxygen control via the stirrer (cascade).	Switch off the cascade and test operation via the <i>Stirrer</i> parameter.	Operator

Interference		
Unusual sounds when the stirrer is switched on.		
Possible cause	Remedy	By
Stirrer is in contact with other vessel components, e.g. sensors.	Stop the Batch (process) and switch off the device. Correctly mount the components in the culture vessel and test stirrer with water in the vessel. If interference persists, contact your INFORS HT representative.	Operator

Interference		
Motor control is volatile, irregular or stops.		
Possible cause	Remedy	By
The motor cable was plugged out when the basic unit was switched on.	Replace the motor.	INFORS HT service technician or licensed dealer

**Interferences**

**12.3 Interferences Temperature Control System**

<b>Interferences</b>		
No temperature control.		
<b>Possible cause</b>	<b>Remedy</b>	<b>By</b>
Temperature control is not activated.	Switch parameter <i>Temperature</i> on.	Operator
Stirrer is not activated and/or setpoint = 0.	Switch parameter <i>Stirrer</i> on and enter setpoint > 0, as required.	Operator

<b>Interference</b>		
No cooling or inadequate cooling.		
<b>Possible cause</b>	<b>Remedy</b>	<b>By</b>
No water supply or inadequate flow.	Check the water supply and turn the supply tap if necessary.	Operator
Temperature sensor is not inserted.	Insert the temperature sensor into the immersion pocket in the vessel top plate.	Operator
Cooling lines are blocked due to lime-scale.	Decalcify the device ("Cleaning and Maintenance", "Decalcifying the Device"). If interference persists, contact INFORS HT representative.	Operator
Ambient temperature in laboratory too high (requirements see "Technical Data", "Operating Conditions".) or/and device(s) with high heat radiation in the immediate vicinity.	Reduce room temperature and/or increase air circulation. Reposition the device.	Operator

## Interferences

### 12.4 Interferences Gassing System

Interferences		
No gassing / air bubbles in the culture vessel.		
Possible cause	Remedy	By
The on-site gas supply has been interrupted.	Stop the Batch (process). Check the on-site gas supply and switch it on, if necessary.	Operator
<i>Flow</i> parameter(s) is/are not activated. And/or: Setpoint in the <i>Flow</i> parameter(s) = 0 (zero). Or: The <i>TotalFlow</i> = 0 and/or <i>GasMix</i> parameter(s) is/are not activated.	Activate <i>Flow</i> parameter(s).  And/or: Set the setpoint in the <i>Flow</i> parameter(s) > 0 (zero).  Or: Set the <i>TotalFlow</i> parameter > 0 (zero) and activate <i>GasMix</i> .	Operator
Hose connection(s) between the basic unit and the culture vessel is/are kinked or clamped.	Check whether the hose connection(s) is/are clamped, if necessary open the clamp(s). Check hose connection(s) for kinks, if necessary route them again or replace them under observation of the sterility requirements.	Operator
Inlet air filter blocked.	Replace the inlet air filter under sterile conditions.	Operator
Exit gas filter blocked.	The overpressure sensor switches gassing off for 10 s, replace the exit gas filter under sterile conditions.	Operator

Interference		
Overpressure alarm <i>Gas pressure high</i> is displayed, the desired gas flow rate is not reached.		
Possible cause	Remedy	By
Blocked holes on the sparger.	Stop the process (Batch), clean the sparger.	Operator
Inlet air filter blocked	Replace the inlet air filter under sterile conditions.	Operator
Exit gas filter blocked	The overpressure sensor switches gassing off for 10 s, replace the exit gas filter under sterile conditions.	Operator

**Interferences**

<b>Interference</b>		
Sudden increase in evaporation losses in the culture vessel.		
<b>Possible cause</b>	<b>Remedy</b>	<b>By</b>
The exit gas cooler does not cool, the <i>Temperature</i> parameter is activated.	Check the water supply to the exit gas cooler, restore it if necessary. The exit gas cooler or basic unit is calcified. Decalcify the device, if necessary.	Operator
The exit gas cooler does not cool. The control valve for water flow is closed.	Open the control valve.	Operator

**12.5 Interferences pH-System**

<b>Interference</b>		
No display or incorrect display of pH, the message <i>ERROR</i> is displayed instead of the current value		
<b>Possible cause</b>	<b>Remedy</b>	<b>By</b>
Sensor cable not connected or not properly connected.	Connect properly if necessary.	Operator
pH drift during long cultivation.	Recalibrate pH with offline values ("Product Calibration", see main chapter "Operation").	Operator
Faulty pH-sensor.	Test calibration with pH 4 and pH 7 buffer. Note the error message ( <i>Show Sensor Status</i> ) when calling up the calibration menu Regenerate or replace the sensor. Consult the documentation of the sensor manufacturer!	Operator

## Interferences

Interference		
No pH control.		
Possible cause	Remedy	By
The <i>pH</i> parameter is not activated.	Activate the <i>pH</i> parameter.	Operator
Pumps are not switched on.	Switch on pump1 ( <i>Acid</i> ), pump2 ( <i>Base</i> )	Operator
Incorrect dead band setting.	Check the dead band (Dead Band in PID settings): Switch off or enter a small value.	Operator
No addition of reagents (acids and base).	Check the reagent bottles: Refill if necessary. Check the hose connections between the reagent bottles and the vessel: Connect properly if necessary. Remove clamps if necessary.	Operator
Pump (base/acid) does not operate properly.	Check the pump (acid/base) functionality on the operating panel.	Operator
Pump hose is damaged. Pump does not rotate: Faulty pump head.	Replace pump head.	Operator

Interference		
pH value drifts up and down over time or acid and base are added almost continuously in turn.		
Possible cause	Remedy	By
Incorrect PID setting in <i>pH</i> parameter.	Check the PID settings and adjust as necessary. Change the special proportional factor ( <i>Prop. Term</i> ) or <i>Dead band</i> setting.	Operator
Incorrect strength of reagents: Concentration is too weak or too strong.	Check the strength of reagents. Adjust if necessary: 0.1 mol to 2.0 mol.	Operator

**Interferences**

**12.6 Interferences pO<sub>2</sub> System**

<b>Interferences</b>		
No display or incorrect display of pO <sub>2</sub> , the message <i>ERROR</i> is displayed instead of the current value.		
<b>Possible cause</b>	<b>Remedy</b>	<b>By</b>
Sensor cable not connected or not properly connected.	Check the sensor cable, connect it properly if necessary.	Operator
Faulty pO <sub>2</sub> sensor.	Check the calibration of the pO <sub>2</sub> sensor. Note the error message ( <i>Show Sensor Status</i> ) when calling up the calibration menu. Replace the sensor if necessary. Consult the documentation of the sensor manufacturer.	Operator

<b>Interference</b>		
No pO <sub>2</sub> control.		
<b>Possible cause</b>	<b>Remedy</b>	<b>Remedied by</b>
The pO <sub>2</sub> parameter and/or cascaded parameter is/are not activated.	Activate parameters.	Operator
The cascade settings are incorrect.	Check the cascade settings and change as necessary.	Operator.
No gas flow into culture vessel.	See faults in the gassing system.	Operator
Fault with control of gas mixing unit.	Check connections. Check gas lines.	Operator

<b>Interference</b>		
Unstable pO <sub>2</sub> control		
<b>Possible cause</b>	<b>Remedy</b>	<b>Remedied by</b>
Incorrect PID settings in the pO <sub>2</sub> parameter.	Check the PID settings ( <i>PID</i> parameter option) and adjust as necessary. Special proportional factor ( <i>Prop. Term</i> ) and dead band. Dead band value must be 0 (zero).	Operator

## Interferences

### 12.7 Interferences Antifoam/Level Sensor and Antifoam Pumps

Interference		
Foam/medium is not detected.		
Possible cause	Remedy	By
Sensor is not properly connected.	Check connections and connect properly as necessary.	Operator

Interference		
Foam/medium is always/frequently detected.		
Possible cause	Remedy	By
Sheathing of antifoam sensor is damaged.	Have the sheathing of the antifoam sensor replaced.	INFORS HT service technician or licensed dealer

Interference		
Antifoam pump does not work.		
Possible cause	Remedy	By
The <i>Foam</i> parameter is not activated.	Activate the <i>Foam</i> parameter.	Operator
Pump 3 ( <i>antifoam</i> ) is not switched on.	Switch on pump 3 ( <i>antifoam</i> ).	Operator

Remedy		
No antifoam agent or medium supply or inadequate flow.		
Possible cause	Remedy	Remedied by
Reagent bottle is empty.	Refill if necessary.	Operator
Incorrect antifoam agent or incorrect concentration.	Replace if necessary.	Operator
Hoses blocked or clamped.	Check the hose connection between the reagent bottle and the culture vessel: If necessary, connect them correctly. Remove clamps if necessary.	Operator
The corresponding pump is not functioning correctly.	Check the function of the pump using the operating panel.	Operator
The pump hose is damaged.	Replace pump head.	Operator
Incorrect hose type connected.	Replace if necessary.	Operator

## 12.8 Interferences Addition of Nutrient Solution (Feed Pump)

Interference		
No addition or inadequate addition of nutrient solution.		
Possible cause	Remedy	By
The <i>Feed</i> parameter (pump) is not activated.	Activate the <i>Feed</i> parameter (pump).	Operator
<i>Feed</i> parameter (pump) setpoint = 0.	Set <i>Feed</i> parameter (pump) setpoint > 0.	Operator
Hose lines blocked or clamped.	Check the hose connection between the reagent bottle and the culture vessel: If necessary, connect them correctly. Remove clamps if necessary.	Operator
Reagent bottle empty.	Refill if necessary.	Operator
<i>Feed</i> pump is not functioning correctly.	Check the function of the feed pump on the operating panel.	Operator
The pump hose is damaged.	Replace pump head.	Operator
Pump head does not rotate: Defective pump head.	Replace pump head.	Operator
Incorrect hose type connected.	Check the hose type. Replace if necessary.	Operator

## 12.9 Replacing Device Fuses

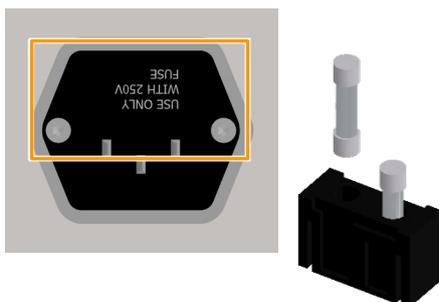


### INFORMATION

Device fuses may only be replaced by fuses of the same rating. For detailed information concerning the requirements for the fuses refer to main chapter "Technical Data", chapter "Connection Values", "Electrical"

To replace a defective device fuse, proceed as follows:

### Procedure



1. Switch off the device and pull out the power plug.
2. Unlock the plug for the fuses by pressing together the two flaps and pull out the plug at the same time.
3. Remove the defective fuse.
4. Insert a new fuse with the correct number of Amperes.
5. Push the plug as far back in the opening as possible until it snaps in.
6. Re-establish the power supply to the device.

## Interferences

### 12.10 Behaviour in Case of Power Interruption

If the power supply to the device is interrupted during a running cultivation process (e.g. by turning off at the power switch or in case of a power failure), all parameter setpoints are stored.

After the power supply is restored, an interrupted cultivation process is automatically continued with the last stored setpoints.

The fact that a power interruption has occurred is indicated by the system alarm *Restart after power failure*. However, the duration of the event cannot be determined from the alarm.

### 12.11 Returning for Repair

The provider must return the device or the faulty component part(s) to the manufacturer if, after consulting the service department of the local dealer or the manufacturer, on-site diagnosis and/or repair is not possible.



#### INFORMATION

When returning the device, the component part or accessory for repair, it is required for the safety of all parties involved and because of legal provisions that a lawful declaration of decontamination is present. Refer to main chapter "Safety and Responsibility", chapter "Declaration of Contamination" for details.

## 13 Disassembly and Disposal

The device must be disassembled and disposed of in an environmentally friendly manner if it is no longer in use.



### INFORMATION

When returning the device for disassembly or disposal, it is required for the safety of all parties involved and because of legal provisions that a lawful declaration of decontamination is present. Refer to main chapter "Safety and Responsibility", chapter "Declaration of Contamination" for details.

### 13.1 Disassembly

Prior to disassembly:

- Switch off the device and lock any isolation switch in the 'off' position.
- Physically disconnect the main energy supply from the device and wait for components to fully discharge.
- Remove and dispose of all additional consumable items, auxiliary components and/or spent processing material in an environmentally acceptable manner.

Clean and disassemble component parts professionally with regard to any local regulations concerning employment and environmental protection. If possible, separate materials.

## Disassembly and Disposal

### 13.2 Disposal

Recycle disassembled components if no agreement is made concerning reclaim or disposal.

- Send metals for scrap.
- Send plastic components for recycling.
- Sort and dispose of the remaining components according their material composition.



#### **WARNING**

Electronic waste, electronic components, lubricants or other auxiliary materials/supplies are subject to hazardous waste regulations and may only be disposed of by registered specialist disposal firms.

For disposal, the system units are to be disassembled and dismantled into individual material groups. These materials are to be disposed of according to the applicable national and local legislation.

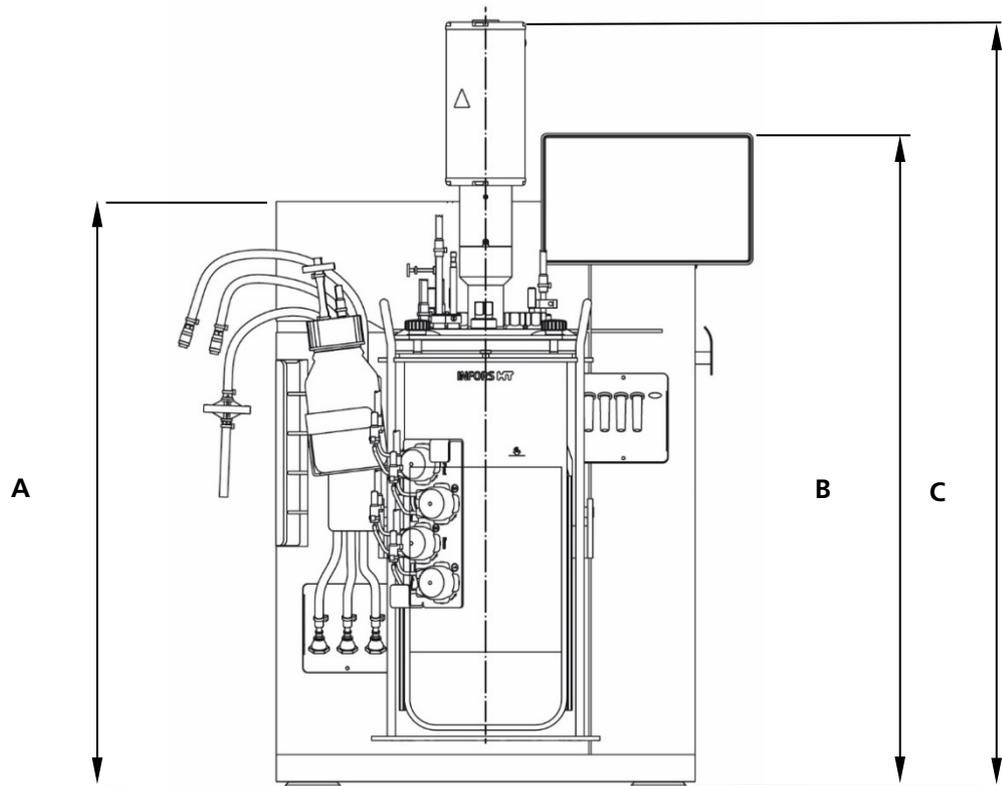
Local authorities or specialist disposal firms can provide information regarding environmentally acceptable disposal.

If no special arrangements have been made for return, INFORS HT units with the required declaration of decontamination can be sent back to the manufacturer for disposal.

## 14 Technical Data

### 14.1 Dimensions

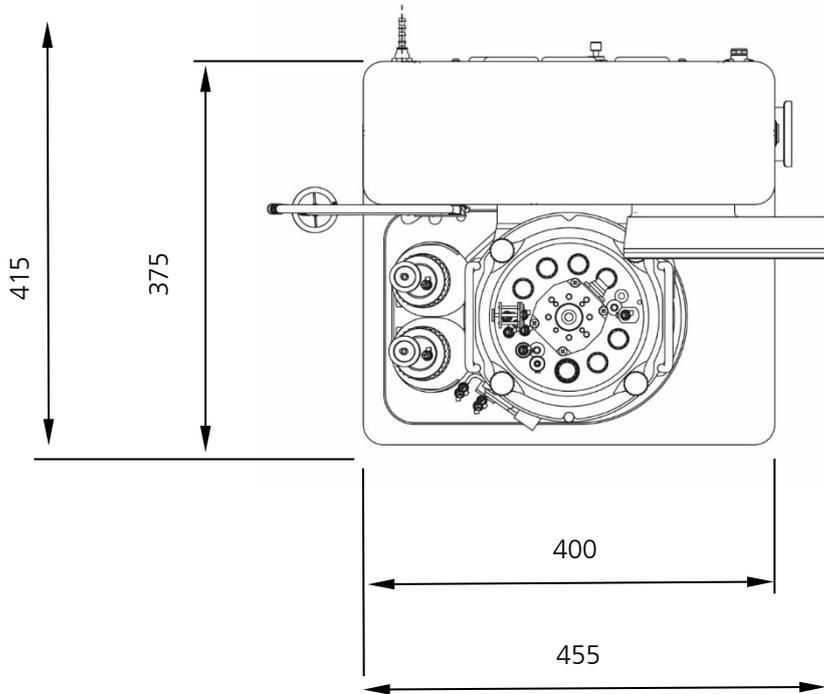
#### 14.1.1 Front View Device



Description	Value	
A	565 mm	Both device versions
B	631 mm	
C	740 mm	Culture vessels NW115 & NW145 for microorganisms
	770 mm	Culture vessel NW90 for microorganisms
	815 mm	Culture vessels, all sizes for cell culture

## Technical Data

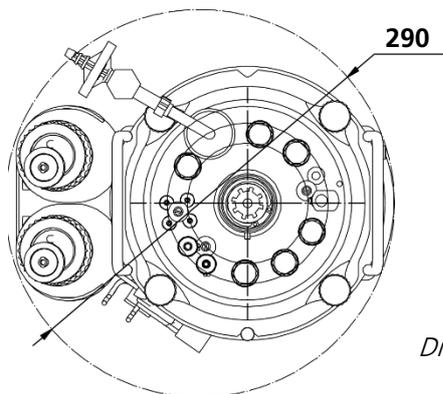
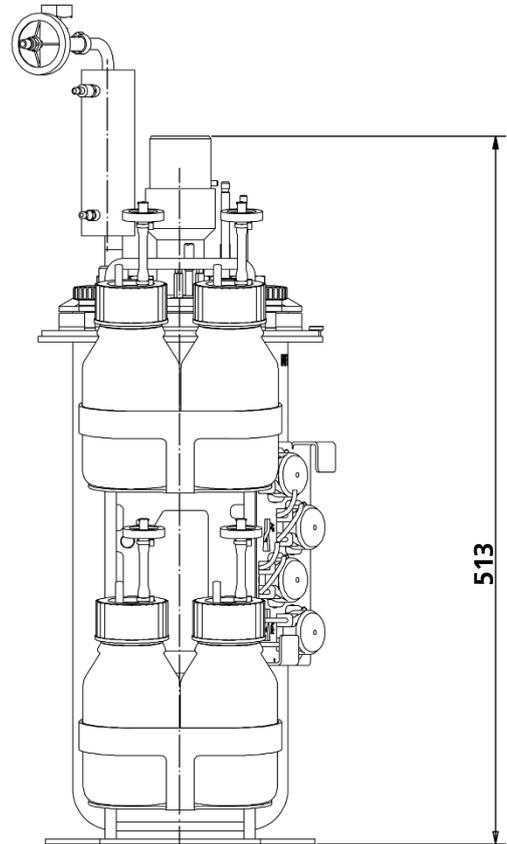
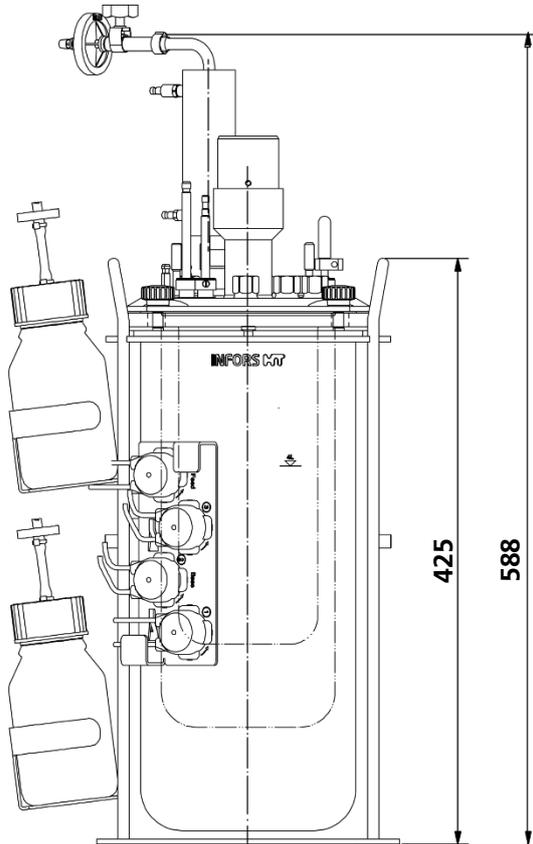
### 14.1.2 Top View Device



*Dimensions in mm*

### 14.1.3 Culture Vessel

The two dimension drawings show a fully equipped culture vessel ready for autoclaving.



*Dimensions in mm*

## Technical Data

### 14.2 Weights (netto)

Basic unit	Culture vessel <sup>1)</sup>		
	NW 90	NW 115	NW 145
23.5 ± 0.5 kg	6.0 ± 0.5 kg	7.0 ± 0.5 kg	9.0 ± 0.5 kg

<sup>1)</sup> Equipped culture vessel, without medium, with vessel holder. The actual weight depends on design and allocation.

### 14.3 Connection Requirements

#### 14.3.1 Electrical

Description	Value	Unit
Voltage	120 / 230	VAC
Frequency range	50 / 60	Hz
Max. current	8	A
Max. power consumption <sup>1)</sup>	~ 800	W
Fuses (5 x 20 mm, slow-blown)	8	A

<sup>1)</sup> During heating phase, vessel with max. 4 L working volume, at max. rotation speed.

#### 14.3.2 Water

##### Water inlet basic unit

Description	Value	Unit
Connection pressure	2 ± 1	bar
Hose nozzle connection, nominal width	6	mm
Water quality	"Very soft" / "soft" (CaCO <sub>3</sub> concentration: 0 mmol L <sup>-1</sup> to 1.5 mmol L <sup>-1</sup> )	

##### Water outlet basic unit

Description	Value	Unit
Connection pressure	No back pressure	
Hose nozzle connection, nominal width	6	mm

**Technical Data**

**14.3.3 Gas**

Description	Value	Unit
Constant connection pressure	2 ± 0.5	bar
Hose nozzle connection, nominal width	6	mm
General gas quality	Dry, clean and free of oil and dust	
Recommended compressed air quality	Class 1,2,3,4 as per DIN ISO 8573-1	

*These specifications apply to all used gases except for the stated recommended quality of compressed air.*

**14.4 Specifications**

**14.4.1 Operating Panel**

Description	Value
HMI	7" colour touch screen
Operating system	Embedded Linux
OPC server	OPC UA

**14.4.2 Culture Vessels**

Description	Value	
Operating pressure in culture vessel	Pressureless	
Form	Cylindrical with flat bottom	
Material	Glass vessel	Borosilicate glass
	Top plate and mounting parts	Stainless steel, AISI 316L, electrolytically polished <sup>1)</sup>
	O-rings (in contact with product)	EPDM

<sup>1)</sup> *Exception: impellers in culture vessel 1.5 L / DN 90 for microorganisms are made of PEEK.*

## Technical Data

### Vessel sizes

TV	Max. WV	Min. WV		DN	Height
		M	C		
1.5 L	1.0 L	0.3 L	0.3 L	90 mm	235 mm
3.0 L	2.0 L	0.6 L	0.7 L	115 mm	295 mm
6.0 L	4.0 L	1.1 L	1.5 L	145 mm	370 mm

Key:

*TV = Total volume / WV = Working volume (maximum and minimum)*

*DN = nominal diameter (inner diameter vessel)*

*M = Microorganismes / C = Cell culture*



### INFORMATION

The volume markings on the glass vessels are only intended as visual aids. They do not represent precise measurements in litres.

### Ports in top plate

Port		Quantity acc. to vessel DN		
Ø	Thread	DN 90	DN 115	DN 145
7.5 mm	None	4	4	4
10 mm	None	4	4	4
12 mm	Pg13.5	4	6	7

**Technical Data**

**14.4.3 Stirrer**

Description	Microorganisms	Cell culture
Drive	Shaft with mechanical seal	
Direction of rotation of drive shaft	Counter clockwise = to left	
Bearing	Outside vessel, in drive hub	
Motore type	DC, brushless	
Nominal power of motor	Small motor <sup>1)</sup> : 102 W Large motor <sup>2)</sup> : 260 W	74 W
Range of rotation speed	150 min <sup>-1</sup> to 1600 min <sup>-1</sup>	24 min <sup>-1</sup> to 600 min <sup>-1</sup>
Accuracy measuring	± 5 min <sup>-1</sup> at 100 min <sup>-1</sup> to 1600 min <sup>-1</sup>  1 % setpoint at > 1000 min <sup>-1</sup>	± 2 min <sup>-1</sup> at 24 to 300 min <sup>-1</sup>  ± 4 min <sup>-1</sup> at > 300 to 600 min <sup>-1</sup>
Accuracy control	1 % Full Scale	

<sup>1)</sup> For vessels DN 90

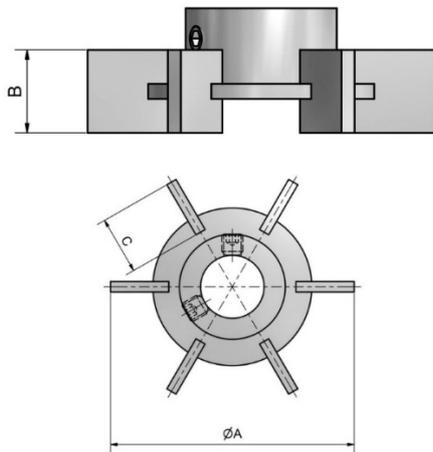
<sup>2)</sup> For vessels DN 115 and DN 145



**INFORMATION**

Ranges of rotation speed are valid in liquid viscosity similar to water, without gassing, with 2 Rushton impellers for version for microorganisms or with 1 pitched blade impeller for version for cell culture.

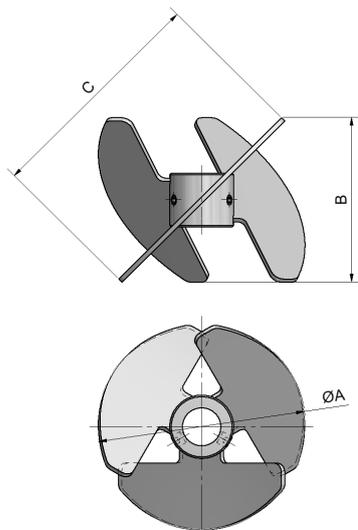
**Technical Data**



**Impellers for microorganisms**

Quantity / type	Material	
	Vessel DN 145 and DN 115	Vessel DN 90
2 Rushton impellers with 6 blades	Stainless steel, 316L, electrolytically polished	PEEK

Vessel	A	B	C
6.0 LTV / DN 145	54 mm	11 mm	11 mm
3.0 LTV / DN 115	46 mm	11 mm	11 mm
1.5 L TV / DN 90	38 mm	9 mm	11 mm



**Impellers for cell culture**

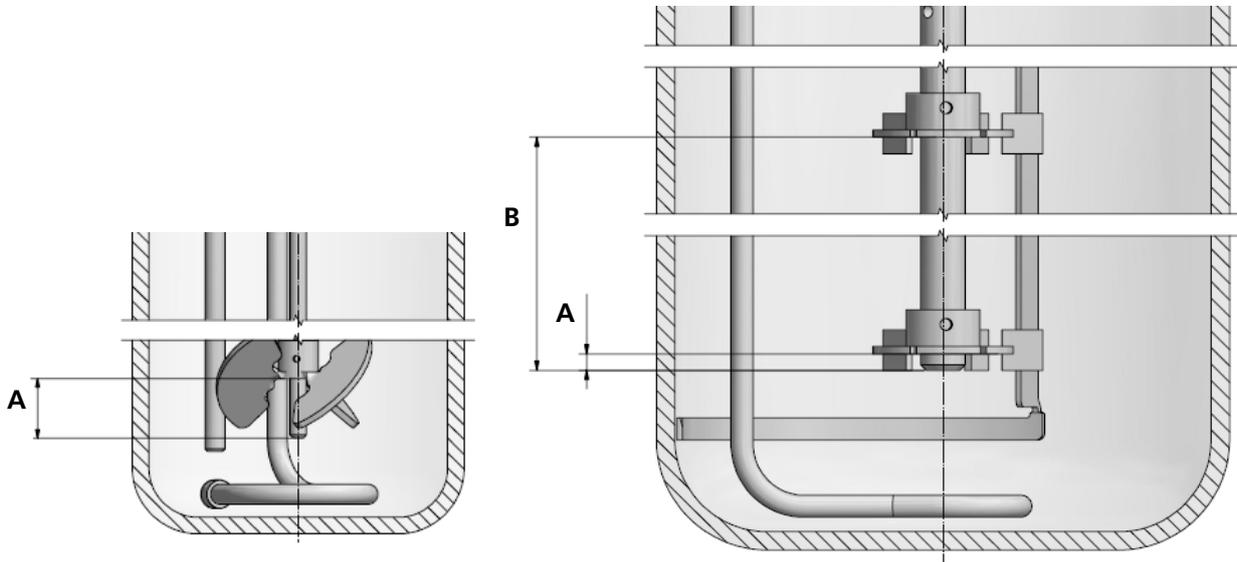
Type	Quantity	Material
Pitched blade impeller with 3 blades, angled 45°	Standard: 1 piece Option: 2 pieces	Stainless steel, 316L, electrolytically polished
<b>Flow direction blades</b> Standard: downwards Option: upwards		

Vessel	A	B	C
6.0 LTV / DN 145	85 mm	65 mm	90 mm
3.0 LTV / DN 115	65 mm	52 mm	72 mm
1.5 L TV / DN 90	50 mm	30 mm	40 mm

**Impeller mounting heights ex-factory**

*Example pitched blade impeller*

*Example Rushton impeller*



Vessel size	Pitched blade impeller	Rushton impellers	
	A	A	B
6.0 LTV / DN 145	16 mm	4,5 mm	137 mm
3.0 LTV / DN 115	17 mm	6,0 mm	110 mm
1.5 L TV / DN 90	18 mm	3,0 mm	89 mm

**14.4.4 Temperature**

Description	Value
Heating	Electrical, thermal block 630 W
Cooling	Tap water <sup>1)</sup> via thermal block and adapter
Sensor	Type: Pt100 1/3 DIN-B
Measuring range	0 °C to +145 °C
Control range	From 5 °C higher than preheating temperature to 60 °C
Accuracy at +20 °C to +60 °C	Measuring: ± 0.1 °C Control: ± 0.2 °C

<sup>1)</sup> A circulating cooler can be used instead of tap water for the cooling system.

## Technical Data

### 14.4.5 Gassing

#### Version for microorganisms

Description		Value
Gas entry		Sparger
Specific gas flow rate, calculated for the max. working volume for all vessel sizes.		20 L min <sup>-1</sup>
Gas(es)	Flow control	Accuracy MFC
Air	MFC <sup>1)</sup> , 2 pieces	± 0.05 L min <sup>-1</sup>
Air + O <sub>2</sub>		
Air + N <sub>2</sub>		

#### Version for cell culture

Description		Value
Gas entry		Sparger Head space <sup>2)</sup>
Specific gas flow rate, calculated for the max. working volume for all vessel sizes.		2000 mL min <sup>-1</sup>
Gasses	Flow control	Accuracy MFC
Air	MFC <sup>1)</sup> , 5 pieces	± 4 mL min <sup>-1</sup>
O <sub>2</sub>		
N <sub>2</sub>		
CO <sub>2</sub>		

<sup>1)</sup> *Mass flow controller, pre-installed*

<sup>2)</sup> *Air and/or CO<sub>2</sub> possible*

#### Control ranges of gas flow

Vessel sizes		Microorgan- isms	Cell culture
Total volume	Max. working volume	L min <sup>-1</sup>	mL min <sup>-1</sup>
1.5 L	1.0 L	0.05 to 2.0	1.5 to 150
3.0 L	2.0 L	0.05 to 4.0	3.0 to 300
6.0 L	4.0 L	0.05 to 8,0	6.0 to 600

**i** INFORMATION

The mass flow controller is calibrated by the manufacturer ex works at standard conditions, i.e. at 1.013 bar and 20 °C. Therefore, for every gas flow rate the gas volume flow is given in L min<sup>-1</sup> and mL min<sup>-1</sup>.

### 14.4.6 pH

**Control system**

Via cascade	Addition of acid and base via peristaltic pumps <i>Acid</i> and <i>Base</i>
	Addition of CO <sub>2</sub> <sup>1)</sup> instead of acid possible.

**Measurement system, digital**

Conventional pH sensor (potential measurement against reference) with built-in electronics

Measurement range	pH 2 to pH 14
-------------------	---------------

<sup>1)</sup> *Version for cell culture only*

**Sensor variants**

Type	Manufacturer
Easyferm Plus ARC	HAMILTON
InPro3253i, ISM, with transmitter M100	METTLER TOLEDO

**i** INFORMATION

pH sensors type Easyferm Plus ARC are preconfigured by the device manufacturer INFORS HT. Replacement sensors must be configured before use.

For details on the technical data, usage and maintenance requirements for the pH sensors, see the separate documentation provided by the sensor manufacturer.

## Technical Data

### 14.4.7 pO<sub>2</sub>

Control system	
Via cascade	Stirrer Gas flow Gas mixture (addition of O <sub>2</sub> or N <sub>2</sub> )
Measurement system, digital	
pO <sub>2</sub> sensor with built-in opto-electronics	
Measurement range	0.05 % - 300 % air saturation

#### Sensor variants

Type	Manufacturer
Visiferm DO ARC	HAMILTON
InPro6860i, ISM	METTLER



#### INFORMATION

The pO<sub>2</sub> sensors are preconfigured by the device manufacturer INFORS HT. Replacement sensors must be configured before use.

For details on the technical data, usage and maintenance requirements for the pO<sub>2</sub> sensors, see the separate documentation provided by the sensor manufacturer.

### 14.4.8 Antifoam

Description	Value
Sensor	Conductive with dosing needle
Control, digital	Pump 3: AF (antifoam)
Range	0 or 100 % (OFF or ON)

**Technical Data**

**14.4.9 Pumps**

Type	Quantity		
Peristaltic	4 pieces		
Hoses	Standard	Option 1	Option 2
Inside diameter	1.0 mm	0,5 mm	2,5 mm
Delivery rate <sup>1)</sup>	3.5 ml min <sup>-1</sup>	1,1 ml min <sup>-1</sup>	16,1 ml min <sup>-1</sup>
Material	PharMed BPT		

<sup>1)</sup> Typical figure with water measured at max. rotation speed

Configura- tion	Default		Alternative setting	
	Function	Mode	Function	Mode
Pump 1	<i>Acid</i>	digital	<i>Feed</i>	analogue
Pump 2	<i>Base</i>	digital	<i>Feed</i>	analogue
Pump 3	<i>AF = Anti-foam</i>	digital	<i>Level</i>	digital
			or	<i>Feed</i>
Pump 4	<i>Feed</i>	analogue	<i>Balance Feed</i>	analogue
			or	<i>Dose</i>

**Operating modes**

- Digital = OFF/ON operation with fixed speed
- Analogue = continuous operation with variable speed.

## Technical Data

### 14.5 Operating Conditions

Description	Value
Ambient temperature	5 °C up to 40 °C
Relative air humidity, non-condensing	20 % up to 90 %
Altitude operating location	max. 2000 m.a.s.l
Degree of pollution (as per EN 61010-1)	2
Min. distance from walls, ceilings and other appliances	150 mm

### 14.6 Emissions

Description	Value	Units
Noise emission	<70	dB (A)

### 14.7 Auxiliary Supplies

pH buffers	Intended use
pH 4.0 pH 7.0	For calibrating the pH sensor

# EG-Konformitätserklärung

EC-Declaration of conformity

Déclaration CE de conformité

INFORS HT

Infors AG, Headoffice, Switzerland  
Rittergasse 27, CH-4103 Bottmingen  
T +41 (0)61 425 77 00  
info@infors-ht.com, www.infors-ht.com

## Gemäss der EG-Maschinen-Richtlinie 2006/42/EG, Anhang II 1 A

In accordance with directive on machinery 2006/42/EC, appendix II 1 A

D'après la directive relative aux machines 2006/42/CE 2006, annexe II 1 A

<b>Hersteller</b> <i>Manufacturer</i> <i>Fabricant</i>	Infors AG Rittergasse 27 CH-4103 Bottmingen
<b>Bezeichnung</b> <i>Designation</i> <i>Désignation</i>	Tischbioreaktor Bench-top bioreactor Bioréacteur de paillasse
<b>Typ</b> <i>Type</i> <i>Type</i>	Minifors
<b>Ab Release</b> <i>From release</i> <i>A partir du version</i>	2.1
<b>Ab Seriennummer</b> <i>From serial number</i> <i>A partir du numéro de série</i>	S-000130198

## Dieses Gerät entspricht den grundlegenden Anforderungen der Richtlinien

This device is in compliance with the essential requirements of directives

Cet appareil est conforme aux exigences essentielles des directives

Maschinenrichtlinie 2006/42/EG  
EMV-Richtlinie 2014/30/EU

Directive on machinery 2006/42/EC  
EMC directive 2014/30/EU

Directive relative aux machines 2006/42/CE  
Directive CEM 2014/30/UE

**Aussteller**  
*Issuer*  
*Editeur*

Bevollmächtigter für die technische Dokumentation  
*Person authorised to compile the technical file*  
*Person autorisée à constituer le dossier technique*

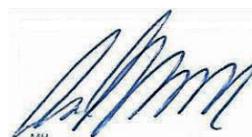


F. Berz

Infors AG  
Rittergasse 27  
CH-4103 Bottmingen

Anschrift  
*Address*  
*Adresse*

Konformitätsbeauftragter  
*Representative for conformity*  
*Responsable de la conformité*



M. Heuschkel  
Chief Technology Officer

Bottmingen, 21. Aug. 2020

Ort, Datum  
*Place, date*  
*Lieu, date*

Digitize your bioprocesses

# The platform software for your bioprocesses



## eve® – the Bioprocess Platform Software

Able to do more than just plan, control and analyze your bioprocesses, eve® software integrates workflows, devices, bioprocess information and big data in a platform that lets you organize your projects in the cloud, no matter how complex they are.

Learn more at [www.infors-ht.com/eve](http://www.infors-ht.com/eve)