LAMBDA MINIFOR Laboratory Fermentor - Bioreactor

Operation Manual

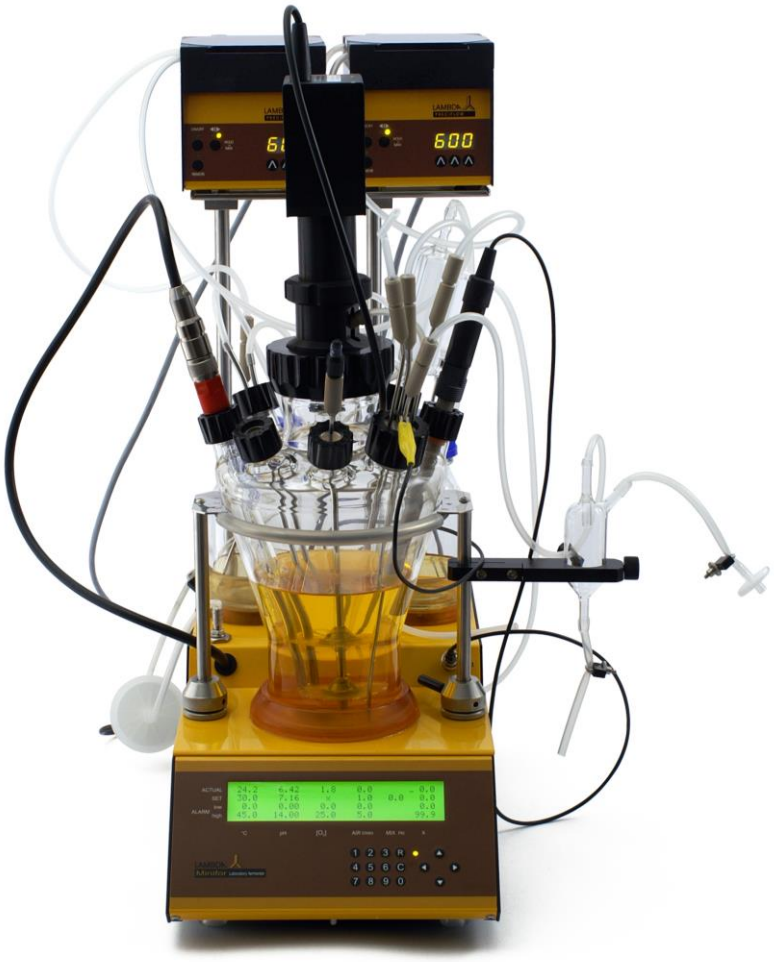

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Table of Contents

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Disclaimer Notice

LAMBDA CZ s.r.o. reserves the right to change information in this document without notice. Updates to the information in this document reflect our commitment to continuing product development and improvement.

Symbols and signs used in the manual:

Tips and tricks message allows learning more about the easy handling of the equipment.

Attention message provides safety instructions, safeguards for you and the equipment. If not followed, it may lead to the damage of the instrument and personal injury.

Thumbs up messages indicate the things that are recommended to be done.

Thumbs down messages forbids the things not to be done with the MINIFOR fermentor-bioreactor.

Background information provides you with the most essential information about the instrument.

Bold Texts found in **“bold”** emphasis keywords or phrases.

1 Delivery check and Inspection

1.1 Check the boxes and the equipment

After receiving the order, inspect the boxes carefully and also the culture vessel and the other parts made of glass for any damages that may have occurred during shipping.

In case of damages, please report to your local purchasing department immediately or send us your claim note. Please note that we can help in claiming the insurance, only when the problem is reported to us within 2 weeks after dispatch.

1.2 Verification of the Delivery note

Kindly check the delivery note and verify that you have received everything and nothing is missing.

If any part of the order found missing, fails to operate or even damaged during shipping, report to us immediately.

2 Introduction

2.1 MINIFOR design concept

LAMBDA MINIFOR fermentor-bioreactor, designed for bench-top surfaces in laboratories is suitable for volumes from as low as ***35 ml to 6.5 l****.*

LAMBDA MINIFOR fermentor-bioreactor has been designed such that several of these can be placed side by side and are well suited for the optimization of bio-transformations and growth parameters of the culture. LAMBDA MINIFOR takes up the minimum amount of space on the laboratory benches and allows having good access to all parts of the unit.

MINIFOR can be used either to work independently or it can be connected directly with a PC to control it remotely and for extensive data treatment, regulation and storage.

Figure 2.1-1 MINIFOR 1L Advanced Kit, front view

Figure 2.1-2 MINIFOR 1L Advanced Kit, side view

Several innovations and new ideas have been introduced,

- For many years, the whole glass vessels have been used in cell cultures and proven to **maintain perfect sterility**, we have used this idea in our fermentor / bioreactor.
- The **agitation system** is based on **up-and-down movement**. The agitator system has a motor with a membrane ensuring sterility and efficient mixing without forming a vortex (thus no baffles are needed). The **bio-mimicking 'fish-tail' stirring discs** offer maximum efficiency without cutting edges; they mix the cells gently and produce less foam.
- The culture is heated by 'sun-like' radiation with a gold reflector and a parabolic radiator under the vessel to maintain the temperature precisely.
- Compact unit, without a casing tower, it is possible to **measure and control six parameters** with the use of its modern microprocessor stored at the front of the instrument.

2.1.1 MINIFOR Advanced and Start-up Kit

LAMBDA MINIFOR fermentor-bioreactor system includes the Advanced and Start-up kit.

Table 1 Short overview of LAMBDA MINIFOR ADVANCED KIT and MINIFOR START-UP KIT

	MINIFOR	
	Advanced Kit	**Start-up Kit**
Reactor scale	Laboratory bench-top fermentor and bioreactor	
Reactor design	Stirred Tank	
Operation mode	batch, fed-batch*, continuous*, (perfusion*)	
Parameter control	stirrer speed, temperature, pH, Dissolved Oxygen, airflow, free parameter, level control for continuous mode.	
*** Optional Parameter control ***	weight control for continuous mode, antifoam, pCO_2, conductivity, REDOX, LUMO (light)	
Microprocessor		
With internal software, display and keypad	**x**	**x**
Agitation		
Automatic stirrer controller **0, 0.1, 0.2, …., 19.9, 20 Hz** **min. 0 Hz (= 0 rpm)** **max. 20 Hz (= 1200 rpm)**	**x**	**x**
Measurement **whole range 0 - 20 Hz**	**x**	**x**
Temperature		
Automatic temperature control **from 5 °C over RT to 70 °C**	**x**	**x**
Measurement with Pt100 **from 0 to 99.9 °C in 0.1 °C steps**	**x**	**x**
IR (infrared) heater	**x**	**x**
Cooling loop	**x**	**x**
pH		
Automatic pH controller	**x**	**x**

0 - 13		
Measurement with pH probe 0 -13	x	x
Acid pump (PRECIFLOW)	x	* optional
Base pump (PRECIFLOW)	x	* optional
Holders and rods for the pumps	x	* optional
Storage vessels and lines for acid and for base	x	* optional
DO (pO_2)		
Automatic DO controller (proportional) 0 - 25 mg Oxygen/L (in 0.1 mg steps)	x	x
Measurement with DO probe 0 - 25 mg Oxygen/L (in 0.1 mg steps)	x	* optional
Airflow		
Automatic aeration controller (proportional) 0 - 5 L/min in 0.01 L/min steps	x	x
Measurement with internal MASSFLOW 0 - 5 L/min in 0.01 L/min steps	x	x
Free Parameter Automatic controller, socket, display	x	x
Alerts For high and low values of each parameter	x	x
Overpressure valve	x	x
Sampling device	x	* optional
Cooling device for outgas/exit gas	x	x
PC-software (FNet, SIAM), Laptop	* optional	* optional
Feed and harvest pump: PRECIFLOW / MULTIFLOW / HiFLOW / MAXIFLOW / VIT-FIT / VIT-FIT HP, pump INTEGRATORS	* optional	* optional
WEIGHING MODULE for continuous mode	* optional	* optional
Automatic Antifoam-system (ANTIFO & DOZITO)	* optional	* optional
O_2-enrichment / 4-gas-mix: controller and massflows MASSFLOW 500 /MASSFLOW 5000	* optional	* optional
Air compressor AEROSILENTO	* optional	* optional
REDOX probe, controller, pump / MASSFLOW	* optional	* optional
PELTIER COOLER for medium / off-gas	* optional	* optional
LUMO light and controller for PBR	* optional	* optional
Operation manual and video	x	x
Warranty	2 years	

LAMBDA MINIFOR is the most flexible fermentor-bioreactor in the market. Initially working with the Start-up kit or even with the Advanced Kit, the system can be upgraded according to the project requirements. For example: changing vessels, adding Pumps and MASSFLOWS, ANTIFO & DOZITO, etc.

2.1.2 LAMBDA Base Control Unit

LAMBDA knows that the biological systems are complex and more the parameters are controlled, the better. Therefore we supply the base control unit that involves measurement of parameters and regulation loops (**°C, pH, pO_2, air flow rate, stirring and another selectable channel X**, which can be used for any other controller. It is ready to accept a **scale module for continuous fermentations**) for precise control of the parameters.

All processes are controlled digitally by two microprocessors. The modern technology allows a much better control than it was used before. Modern technology allows us to gather all the control loops in a small space at relatively low cost and we have used it to make our customer benefit a lot from it, instead of selling each and every module at a high cost.

The base control unit contains the control panel that is used for the display and control of the parameters.

2.1.3 MINIFOR Vessels

MINIFOR can be used to cultivate the minimal working volume of 35 ml with the precise control of 6 parameters!

It has the largest volume range available on the market, because it is the only instrument that can be used for volumes ranging from 35 ml to over 6 l. The base unit will remain the same but the working vessels can be exchanged according to the project requirements. The speed of signal traffic is higher and the regulation is more precise in each and every base unit.

LAMBDA designed and created ports on the sides of the vessels. The access is much better and global overview is very good. The innovative form of the LAMBDA vessel eliminates the expensive head plate completely. The threaded central screw cap allows an easy mounting and opening with just one hand movement.

Table 2 **MINIFOR Vessel Overview**

Vessel type	0.3L	0.4L	1L	3L	7L
Working volume					
Minimum (L)	0.035	0.15	0.3	0.5	1.0
Maximum (L)	0.4	0.45	1.7	3.0	6.5
Vessel dimension for autoclaving					
Height (cm)	34	22	34	37	50
Diameter (cm)	22	23	25	34	30
Ports					
No. of side necks	6	8	8	8	8
≈ traditional ports		22	22	22	22

2.1.3.1 LAMBDA Liquid O-ring for 7L vessels

Standard O-rings have several disagreeable properties.

- If any dirt or filaments are closed under the O-ring, then microbes will have a large tunnel to get into the vessel.
- Large O-rings require large compression forces to be perfectly tight. The screwing of the connection surfaces must be done perfectly and regularly in order to eliminate the higher compression on one side and lower compression on the other side. The resulting mechanical forces may have a high stress on glass parts, especially during the sterilization.
- Frequent replacement of large O-rings may represent high recurrent costs**.**

Therefore, for the large 7 L vessels, LAMBDA proposes a new solution to the above mentioned problems, the **liquid O-ring**:

The liquid O-ring **consists of a new partial hardening silicone resin filled with the purest quartz powder of extremely fine (fume like) consistency**.

It fills the cavities between the joining surfaces**,** is completely gas tight and also does not generate any stress on the glass.

It will either cure in several hours at room temperature or within minutes in the autoclave during sterilization. The liquid O-ring forms a large surface joint and not just a few mm with a large contact **surface to cover** as in the case of standard O-rings.

Moreover, this liquid **O-ring paste** can be **reused** many times.

2.1.4 Agitation unit

The LAMBDA agitation unit is included in each MINIFOR kit.

LAMBDA uses a non-rotational agitation system with up and down movement. This agitation system shows a new possibility to achieve an easy and perfect sterility, maintained only with an expensive magnetic coupling. The problems caused by the magnetic coupling are totally eliminated here and combined with the agitation system used.

The **vibromixer**: a strong motor moves one or more stirring discs up and down.

The frequency of agitation is controlled by a microprocessor. The stirrer range is **0 – 20 Hz** and can be chosen in steps of **0.1 Hz**.

The stirrer frequency as well as the amount and type of stirrer discs can be choosed according to the applications.

Available stirrer discs are:

- Standard fish-tail stirrer discs: for mammalian cells, plant cells, fungi, bacteria, yeast. The softest agitation and biomimetic 'fish-tails' ensures uniform mixing throughout the fermentation / bioreaction.
- Optional metal stirrer discs: for viscous medium
- Butterfly stirrer discs for low working volume (for example: 35 ml)

2.1.5 Temperature and pH probe

The temperature probe (Pt100) is incorporated with the pH probe. The pH/Pt100 probe is included in each MINIFOR kit.

The Pt 100 is used for

- Automatic temperature correction for the corresponding pH measurement
- Measurement of the temperature of the fermentation medium / cell broth
- Automatic temperature control of the fermentation medium / cell broth.

The pH probe is used for

- Measurement of the pH.

2.1.6 pH control

Each MINIFOR kit contains the microprocessor for the automatic temperature and pH control. The MINIFOR ADVANCED KIT includes 2 PRECIFLOW peristaltic pumps, lines and storage vessels for an automatic pH control.

For an automatic pH control, the microprocessor compares the actual pH value with the set-point. According to the difference of those values, the acid or base will be added to the medium.

Liquid base and/or liquid acid are added automatically by the LAMBDA peristaltic pumps according to the difference between set-point and actual measurement. Thus, it is not done by the on/off but over the whole speed range of the pump.

Instead liquid acid, it is also possible to add gases like: CO_2 for the automatic pH control. Then, it is necessary to disconnect the peristaltic pump for acid and connect the optional MASSFLOW gas controller for the automatic pH control.

2.1.7 Temperature control

Heating blocks as well as silicone heating pads have their well-known disadvantages.

- Their efficiency is limited by the quality of the contact between the heating surface and the vessel walls. The transmission of heat occurs only by diffusion, which is slow especially when the temperature gradient is small.
- Further disadvantage is that the view into the vessel is restricted and it is not possible to illuminate cultures of algae from outside.
- An even worse criterion is the overheating of cell culture, which occurs when the level of the culture liquid is below the upper part of the pad.
- Another major disadvantage is that heating clamps and heating mats prevent natural cooling of the vessel and a complementary cooling is then required. This increases both the cost and complexity of the system. Even a small damage in heating mats can lead to electrocution.

To eliminate all the above mentioned problems in using the heating blocks and pads, LAMBDA invented an **IR radiation heater** which is placed under the bottom of the vessel.

The heating spiral is placed in a gilded parabolic reflector, which concentrates the energy at the bottom of the vessel. The heat rays optimally heat the medium from underneath. This creates a natural upwards convection even without any mechanical agitation and **without creating any hot spots at any medium volume in the vessel**. About a half of the heat is adsorbed by the glass bottom and about a half of the energy is absorbed directly in the medium. It is not possible to imagine a softer sun-like way of heating.

The change of temperature is much faster and its setting and adjustment is more precise and simple to handle.

The temperature is measured with the **Pt100** which is incorporated with the pH probe (please refer **2.1.5 Temperature and pH probe**).

If it is necessary to work with temperatures lower than 4°C above ambient temperatures or if cooling is needed due to the exothermic reaction, then the range of temperature control can be shifted by the addition of the optional cooling system.

The MINIFOR kit includes the **cooling loop** which allows the circulating cooling liquid (for example: tap water or cooling liquid from the thermostatic water bath) to maintain the temperature. Or it is also possible to get the **electronic PELTIER Cooling device** for the working volumes up to 3L

2.1.8 Aeration and pO_2 Control

LAMBDA MASSFLOW controller system is specially designed to use with LAMBDA laboratory bioreactors and fermenters for the measurement and control of air and other gases.

A high quality laminar mass flow sensor measures the flow rate. The result appears on the digital display of the base control unit. The mass flow cell has a very low pressure drop and a linearity error less than ±3% reading. The repeatability is better than ±0. 5% reading.

The flow rate is regulated by a special proprietary proportional needle valve controlled by a microprocessor. The flow rate can be programmed and volume can be calculated.

Initially the parameter needs to be chosen for the control:

- Automatic flow-control [L/min] of one gas (mix)
  or
- Automatic control of pO_2 (DO, dissolved Oxygen) [Please refer **chapter 5.3**]

2.1.8.1 Air flow

Before using the gas-flow (L/min), it's a must to branch the pressured gas in terms of 0.05.

Each MINIFOR kit contains an **internal MASSFLOW**: 0 – 5000 ml/min, control in 10 ml/min steps. The proportional valve for the gas supply opens according to the set-point.

The internal MASSFLOW of MINIFOR can be used for one stream of gas or one stream of gas-mix.

For gas mix and O_2 enrichment, optional **external MASSFLOWs** can be used**:**
- External MASSLFOW 5000: 0 – 5000 ml/min, control in 10 ml/min steps
- External MASSFLOW 500: 0 – 500 ml/min, control in 1 ml/min steps

2.1.8.2 pO_2 (DO = dissolved Oxygen) probe

By adding air/O_2, the internal MASSFLOW (0-5000 ml/min, control in 10 ml/min steps) can also be used for automatic pO_2 control.

Furthermore, the pO_2 probe (OD probe = dissolved Oxygen probe) is required.

The MINIFOR ADVANCED KIT is equipped with the DO probe. It is possible to get the pO_2 probe to be used with the MINIFOR Start-up Kit, if needed.

Technical properties of the DO probe:

- pO_2 probe can be sterilized up to 130°C.
- Short response time, less than 1 min. to 95 % of end signal
- Wide measuring range 0-25 mg dissolved oxygen/l
- Automatic temperature compensation
- Process pressure up to 3 bars
- Polarization time less than 2 hours

LAMBDA has developed an oxygen probe using a completely non-metal body made of a new**,** very resistant material**,** PEEK, which has a similar chemical resistance to PTFE, but it is mechanically much more stable.

The electrode is of a Clark type with a large Pt cathode and Ag/Cl reference anode. The membrane is made from glass reinforced with a thin layer of PTFE. The PTFE is better than silicone membranes because much less deposits form on the Teflon surface than on any other material.

The membrane is almost fully protected and only a very small opening above the cathode is free. The probability of damaging the membrane is therefore greatly reduced.

Principle of operation: The PTFE membrane is permeable to gases and will not let any other dissolved substance to pass through. By selection of the right polarization voltage, the oxygen, which diffuses through the membrane**,** is reduced on the cathode and the electric current proportional to the oxygen concentration is generated. This signal is measured and transformed to the concentration of oxygen dissolved in the medium.

2.1.9 REDOX Potential measurement

The measurement of the RedOX potential for the anaerobic culturing can be done with the REDOX probe and the control unit.

REDOX probe is an optional tool that can be obtained if needed.

The measurement of Red-Ox potential is done by using a sterilizable combined pH/temperature probe with an additional Pt electrode fixed on its glass body. This probe is connected to the MINIFOR fermenter-bioreactor in the same way as that of the standard pH probe.

LAMBDA REDOX allows the measurement of the Red-Ox potential and the digital transfer of the data to the PC with the help of the fermentation software SIAM.
The control unit displays the measured RedOx potential in terms of mV.

The output RedOx signal can be received at the “PUMP” socket at the rear of the MINIFOR laboratory fermentor-bioreactor base unit.

2.1.10 Sampling device

The MINIFOR sampling device is included in each MINIFOR ADVANCED KIT.

To decrease the risk of contamination during sample withdrawal, LAMBDA supplies an easy to use sampling device. It consists of a glass trap with three inlet/outlet tubes. Out of which one tube is connected with the sampling port, second has to be connected with the air filter and the third is the outlet that is used for the sample withdrawal.

Samples can be taken from the vessel using one of the cannula from the quadruple sampling assembly (the one with the longest needle).

If needed, the MINIFOR standard sampling device can be replaced by any other sampling device (like the standard in your laboratory).

2.1.11 Antifoam Control

The foam level control system is an optional tool and not included in the MINIFOR Start-up and Advanced Kit.

The LAMBDA MINIFOR laboratory bioreactor-fermenter can be equipped with a novel foam detector and control system:

ANTIFO and DOZITO

2.1.11.1 DOZITO = miniature syringe pump for antifoam addition

The space around fermenter/bioreactor vessel is very precious and the amount of antifoam substance used in fermentation and cell culture processes is usually only a few ml.

For this reason, LAMBDA has developed probably the world’s smallest syringe pump system. A special frequently sterilizable 5 ml glass syringe is used for the addition of anti-foam agent.

The magnetic holder of DOZITO can be placed in any convenient place on the upper cover plate of the MINIFOR fermenter/bioreactor base control unit casing**.**

The volume of added anti-foam liquid can be varied from a dozen micro-litres to about 0.3 ml.

Specifications:

Pushing force: more than 20 N
Step length: adjustable from 0 to 4 mm
Protection: electronic in case of blocking
Dimensions: 3.6 x 2.7 x 12.7 cm (L x W x D)
Weight: 230 g

2.1.11.2 ANTIFO = foam detection system and controller of DOZITO

The presence of foam in the reactor vessel is detected by the measurement of electrical conductivity.

Instead of an additional antifoam probe, two cannulas (long and small) from the quadruple sampling port of the LAMBDA MINIFOR fermenter vessel are used. **No additional ports need to be used for the anti-foam detection and control**.

Specification:

Power supply: 12 V DC, 2W (from the pump socket of the MINIFOR fermenter)
Conductivity range: 1 k Ohm to 0.5 M Ohm
Frequency: 4 kHz AC
Measuring voltage: 100 mV AC
Dimensions: 3 x 3 x 16 cm (L x W x D)
Weight: 220 g

2.1.11.3 Automatic prevention of antifoam over-dosage

The addition of too much antifoam agent is detrimental for the oxygen transfer from the air bubbles into the culture medium. The excessive addition of antifoam liquid (anti-foam over-dosage) is avoided by the introduction of a waiting interval of about 20 seconds after the first antifoam agent addition.

The second addition of antifoam agent occurs only when the foam did not disappear during the elapsed waiting interval time. Additionally, the anti-foam dose can also be controlled in the steps of volume by the LAMBDA DOZITO miniature syringe pump.

2.1.12 Outgas condenser

The outgas condenser prevents the condensation of water on the output filter and the resulting blocking of the outgas air flow. Condensed water flows back into the vessel.

This flow-back is important, particularly when working with low volumes for weeks, otherwise the medium would become concentrated and the working volume would decrease.

2.1.12.1 Outgas condenser with cooling liquid

A glass out-gas condenser that can be connected with a cooling liquid (cooling water) is included in each MINIFOR Kit.

2.1.12.2 Electronic outgas condenser

The electronic outgas condenser is an optional tool. It is not included in the MINIFOR Advanced and Start-up kit.

As an alternative to water-cooled exhaust air condensers, LAMBDA offers a Peltier-based outgas condenser with an electronically controlled operating temperature down to 5 °C.

This results in a better removal of moisture from the outgas stream than the water cooled condensers.

Such an electronic condensing system also allows a more efficient and constant cooling **without any cooling water**!

2.1.13 Parameter "X"

The parameter "X" allows the control of any other parameter apart from **°C, pH, pO_2, airflow rate and stirring.** It is an optional parameter control to be utilized when needed.

Usually, for the continuous mode of fermentation, the weighing module (chemostat) can be used as parameter "X" for controlling the addition or removal of the substrate from the reaction vessel.

It is possible to control the conductivity probe, redox potential and also the CO_2 probe as parameter "X".

2.1.14 Quadruple plug box

The quadruple plug box is an optional tool that can be used with the MINIFOR. It is not included in the standard kits LAMBDA MINIFOR ADVANCED KIT or LAMBDA MINIFOR START-UP KIT.

The quadruple plug box consists of four additional 8-pole connectors (two in the rear and two in the front) that can be connected and controlled by the MINIFOR fermentor-bioreactor.

If additional instruments are needed to be used with the MINIFOR, then the quadruple plug box helps to extend the connection for the addition of up to four additional LAMBDA instruments (e.g. pumps, mass flow gas flow controller, weighing module, antifoam controller, etc.)

This plug box provides a power supply and RS-485 line connections.

Up to two quadruple plug boxes can be connected together to further extend the number of available connection sockets.

2.2 LAMBDA MINIFOR Fermentation Modes / Setup / Options

LAMBDA MINIFOR can be used for batch, fed-batch and continuous mode of operation.

In the following, a short overview of the tools that are needed for the different modes of operations will be explained:

Table 3 **Fermentation modes: batch, fed-batch & continuous mode**

Batch *= LAMBDA MINIFOR kit (Advanced or Start-up)*
Fed-Batch *= LAMBDA MINIFOR kit (Advanced or Start-up) + 1 Feed-Pump*
Continuous *= LAMBDA MINIFOR kit (Advanced or Start-up) + 1 Feed-Pump + 1 Harvest Pump + 1 weighing module (Speed of Feed-Pump = Speed of Harvest-Pump = growth rate)*

Batch	 **MINIFOR (Advanced or Start-up Kit)**			
Fed-Batch	 **MINIFOR (Advanced or Start-up Kit)**	 **Feed-Pump (Peristaltic Pump)**		
Continuous	 **MINIFOR kit (Advanced or Start-up Kit)**	 **Feed-Pump (Peristaltic Pump)**	 **Harvest-Pump (Peristaltic Pump)**	 **Weighing Module**

2.2.1 Batch Fermentations

Table 4 **Fermentation mode: Batch** = LAMBDA MINIFOR kit (Advanced or Start-up)

Batch Mode	 **MINIFOR (Advanced or Start-up Kit)**	-	-	-

A conventional mode of fermentation in a closed system is batch fermentation.

The procedure of incubation, after the inoculation with micro-organisms to a sterile nutrient solution, is carried out. Nothing is added to the fermentation process except acid or base to control and maintain the pH. And air (O_2) is controlled in the case of aerobic micro-organisms.

There is a general constant change of the composition of the culture medium / biomass concentration as a result of the metabolism of the cells.

2.2.2 Variable Volume Fed-Batch

Table 5 **Fermentation mode: Fed-Batch** = LAMBDA MINIFOR kit (Advanced or Start-up) + 1 Feed-Pump

Fed-Batch Mode	 **MINIFOR (Advanced or Start-up Kit)**	 **Feed-Pump (Peristaltic Pump)**		

An enhancement to closed batch process where all of the substrate is added at the beginning of the fermentation is the fed-batch fermentation.

This is where the **substrate is added** in small proportions during the fermentation process. These increments are initially small concentrations of the nutrient solution at the beginning of the process and during the production phase in small doses.

There are 2 basic approaches to this mode of fermentation. These are:

- Constant Volume Fed-Batch Culture (Fixed Volume Fed Batch)
- Variable Volume Fed-Batch

Due to the substrate feed, the volume changes with respect to the fermentation time.

Options:

- Medium used in the batch mode can be added.
- Addition of the same concentration of the limiting substrate solution used in the initial medium.
- Addition at a reduced rate of a concentrated limiting substrate solution is used.

2.2.3 Continuous Fermentation

Table 6 Continuous mode = LAMBDA MINIFOR kit (Advanced or Start-up) + 1 Feed-Pump + 1 Harvest Pump
Continuous + 1 Weighing module (speed of feed pump = speed of harvest pump = growth rate)

Continuous Mode				
	MINIFOR kit (Advanced or Start-up Kit)	**Feed-Pump (Peristaltic Pump)**	**Harvest-Pump (Peristaltic Pump)**	**Weighing Module**

The fermentation mode in which there is an open system set up is the continuous fermentation mode.

An equal quantity of the converted nutrient solution with micro-organisms is removed from the bioreactor vessel and a sterile nutrient solution is added to the reactant vessel continuously.

Two terms are used in the case of a homogeneously mixed bioreactor. The first term, known as **chemostat**, in the steady state, the concentration of one substrate is adjusted accordingly to control cell growth. The second term, known as **turbidistat**, the biomass concentration is monitored, cell growth is kept constant and the rate of feed of the nutrient solution is adjusted appropriately.

2.2.3.1 Weighing module

Our goal was to develop an extremely compact, precise and easy to use device, which could be used for continuous fermentation and cell cultures. The scale module is an optional accessory for the LAMBDA MINIFOR laboratory fermenters and bioreactors. It allows keeping the amount of medium constant, independent of the extent of aeration, foaming and stirring.

This is a much more precise method for the control of medium volume than a low cost overflow tubes or similar devices which control only the level of the culture surface. Therefore, it allows an exact evaluation and calculation of culture parameters.

2.3 Safety Instructions

Do follow the safety instructions and precautions while handling the equipment for your own safety and also for the safety of your equipment. It minimizes the potential risks that are caused during the usage.

Follow your laboratory safety precautions!

2.3.1 Glass vessel handling

Glass is still the best material for bioreactor vessels. It does not leak out heavy metal ions like steel or not polymerized chemicals like plastic materials. Therefore, without any compromise LAMBDA supplies only whole glass vessels.

Glass, however, is fragile, so handle it with all necessary care, especially when you wash vessels.

Pay particular attention that **glass parts and vessels can be broken, if not handled with care.**

Protect the glass surface from scratches made by silica or other hard materials.

Always use the safety over-pressure valve on the vessel! This will prevent a pressure increase in case of the blocking of the output filter in case of excessive foaming.

During **autoclaving**, leave always a **ventilation opening** for **pressure compensation!**

Do not screw the screw caps with too much force! It is not necessary and the glass threads may break.

Never heat an empty reactor! The heat radiation is absorbed by the glass walls and their temperature would increase to such an extent, that thermal dilatation would lead to a vessel burst.

During **autoclaving**, **never fill more than 2/3 of the vessel** volume with liquid!

Use brushes with detergent to **remove dirt** and acidic solutions (acetic acid, citric acid or hydrochloric acid for salt deposits).

Always **place a metal washer under the screw-cap** to reduce the necessary screw force!

To facilitate the **insertion of the multiple-seal silicone stoppers into the glass necks** (e.g. probes, cooling loop, cooler, tubing etc.), you can **wet them with a few drops of distilled water**. They will then slide in and out easily.

For **removing the stoppers and probes** you can also add a drop or two of distilled water between the stopper and the glass wall. Move them from side to side while at the same time pulling them out.

It is highly recommended to place an **intermediary vessel between the condenser outlet and the output gas filter**. A small amount of convenient antifoam solution can be placed on the bottom of this bottle. This will destroy the foam should it **enter this additional vessel** and increase the protection of the output filter against clogging.

All glass components are predestined to **get broken at some time**. If breakage is limited to side necks, generally the vessel can be repaired by a glassblower. LAMBDA keeps also a stock of threaded **necks** for repairs. Broken vessels can also be sent to us. Please, contact us for further information.

2.3.1.1 Special precautions for handling 0.3L vessel

The 0.3L vessels are the smallest and the jacketed reactor vessels of the MINIFOR fermentor-bioreactor. Special safety precautions and tips would help to master safe working with the minimum working volume reactor vessels.

The **small jacketed 0.3L reactors** must be used only with the **vessel jacket completely filled with water**. The hose connectors of the jacket are closed with silicone tubing.

While using the small jacketed 0.3L vessel, the **initial temperature may slightly overshoot** after the first start-up of MINIFOR. It is therefore **advisable** to **set the temperature about 4°C lower than the final working temperature**. After the temperature approaches this temperature, set the final working temperature. This will **shorten the time taken for temperature stabilization**. If time is not critical, you may just set the desired temperature and wait until the system equilibrates automatically.

The **vessel jackets** of the 0.3 L vessels **are not intended for cooling** but only for buffering the temperature variations. It can, however, be used with a **circulating thermostatic bath** or for rapid cool-down, if required for **special reasons**. In this case, inactivate the heating on the MINIFOR by setting the temperature to a low value (e.g. 10°C).

Otherwise follow the general safety construction for handing glass reactor vessels, refer the previous 2.3.1 Glass vessel handling!

2.3.2 LAMBDA Double Lock connectors

The LAMBDA DOUBLE LOCK connection system allows safe and easy **connection of tubing** to the vessel. It is made of PEEK. PEEK is a new material similar to PTFE in its extreme chemical resistance and very high melting point (350°C). Therefore, it can be even flamed. PEEK has a much higher mechanical stability and does not “flow” like PTFE. For its superior qualities, it has been selected by LAMBDA despite its very high price.

2.3.3 Power connection of additional instruments

When connected to the MINIFOR system and its RS-485 line, the pumps and external MASSFLOW gas flow controllers MUST NOT be powered by any external power supply. This is because the used 12V voltage of the external

power supply and the MINIFOR power supply may not be exactly the same and the current may flow from one instrument to the other. This could generate problems and damage the instruments. If such a connection is inevitable a diode in the power supply line would be needed.

Also follow all safety rules of your laboratory and common safety rules while handling electrical equipment!

2.3.4 Sterilization

Never autoclave any cable or the MINIFOR console or other electronic devices, otherwise they will be destroyed!

Never use overpressure on the vessel, otherwise it will be destroyed! Therefore make sure, that you leave open at least one line (no tube clamps!) which leads to the headspace (not medium) for pressure balance. Usually, the off-gas line is the best for it.

See also the special safety rules for the vessels in chapter 2.2.1 and sub chapters for handling the glass vessels!

For autoclaving use slow cooling down / pressure balance as it is usually programmed for “liquid sterilization”

2.3.5 Pressure

Never overpressure the vessel, otherwise it will be destroyed!

Refer the special safety rules for sterilization on chapter 2.3.4

2.3.6 Dangerous reagents and microbes

While handling corrosives and other dangerous reagents put on goggles and gloves and follow your laboratory safety instructions!

While handling pathogens and dangerous microbes apply the necessary safety precautions according to the law / your laboratory zone!

While handling pathogens and dangerous microbes apply the necessary safety precautions according to the law / your laboratory zone!

Do not use HCl as an acid for your fermentation as far as your process allows others like H_3PO_4 or H_2SO_4.

3 Installation and Set-up (based on MINIFOR 1L Advanced Kit)

3.1 Essential things for MINIFOR Setup and Installation - OVERVIEW

Make the workplace ready, to start the MINIFOR Installation.

Table area	22 x 40cm (W x D)
Power supply	Mains 100-240 V AC/50-60Hz, 510W, CE conform
Ambient Temperature	0 – 40 °C
Relative humidity	0 - 90 % RH (non-condensing)
Supply of Air or Oxygen (Aerobic Culture)	0.05 – 0.2 MPa
Supply of N_2 or other gas (Anaerobic Culture)	0.05 – 0.2 MPa
pH calibration	Standard solutions, 2 Buffers according your calibration range (standard pH 7.0 and pH 4.0)
pO_2 calibration	Standard gases: Air for maximum, N_2 for minimum Control gel for pO_2 = 0 mg/L
Sterilization of the vessel and other needed parts	Autoclave
Optional: Maintaining reduced temperature with cooling loop	Cooling water circulation or a thermo stabilized water bath circulation

Please refer to the safety precautions in the chapter 2.3

3.2 Get ready with the LAMBDA Base Control Unit

Initially for starting the installation process, get ready with the LAMBDA base control unit on which the other parts need to be fixed for working. The base control unit includes pH and temperature probe connectors, IR heating coil, MASSFLOW and other microprocessors that control all the parameters that need to be measured and controlled.

Place the base control unit on the work place where the complete installation of MINIFOR Bioreactor will be made.

Figure 3.2-1 The front view of the LAMBDA MINIFOR Base control Unit.

3.3 Holders and Support Rods for LAMBDA Peristaltic Pumps

For the Advanced Kit (which includes two LAMBDA PRECIFLOW Peristaltic Pumps), a setup for placing the pumps and sample bottles has to be made in the MINIFOR base unit which involves the easy and controlled addition of, for example: acid, base, medium, nutrients, minerals, etc. into the working reaction vessel.

Figure 3.3-1 As a first step, insert the support rods in the present basic control unit as shown. Rotate it clockwise for fixing.

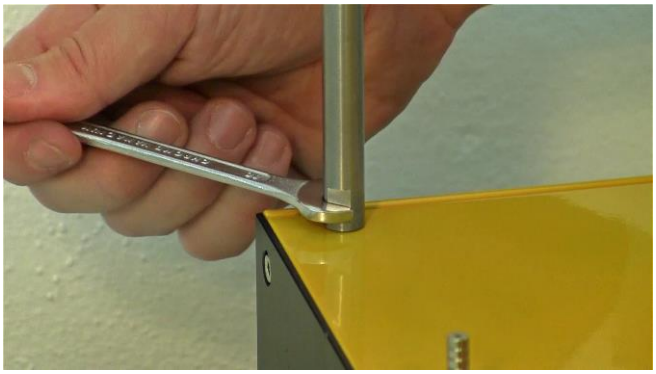


Figure 3.3-2 Further fix the inserted support rod tightly with the supplied spanner.

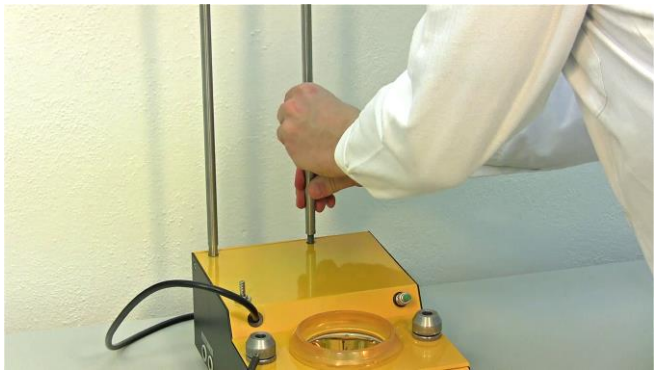


Figure 3.3-3 Insert the second support rod and fix it. So now both the support rods are in place.

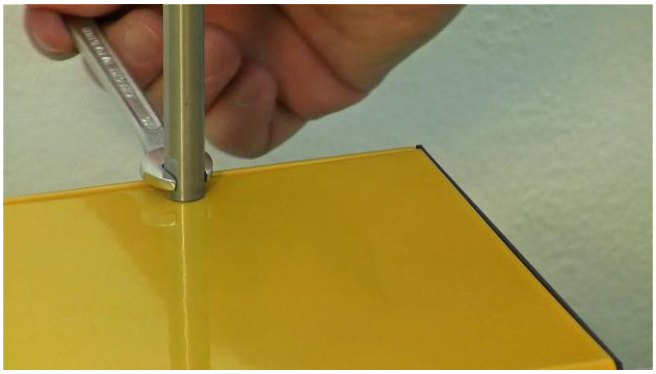


Figure 3.3-4 Fix the second support rod tightly with the spanner like before.

Figure 3.3-5 The adjustable support plate that needs to be inserted on the support rods. (N.B: In case, the LAMBDA Peristaltic pumps are used, for example: controlled addition of acid and base to maintain the pH).

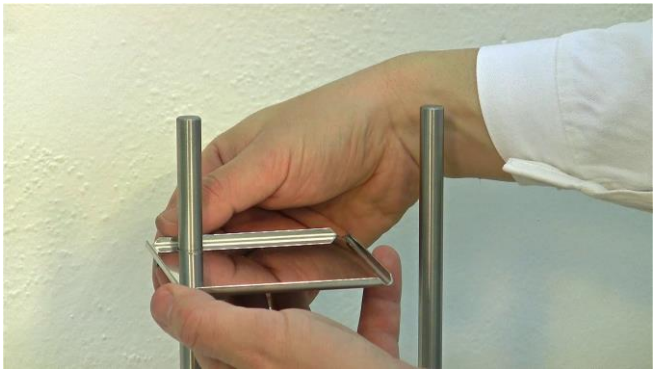


Figure 3.3-6 Insert the adjustable support plate on one of the fixed support rods and fix it tightly using the screws provided for tightening. The support plate can be used for supporting, for example, a LAMBDA Peristaltic Pump.

3.4 Setting up of the Fermentation Vessel

The fixing of the support rods and support plate for peristaltic pump has to be followed by the installation of the holders for fermentation vessel and sterile sampling device.

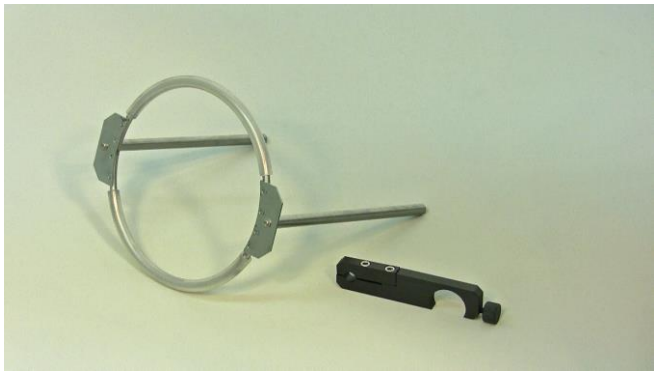


Figure 3.4-1 Reaction vessel holder and the sterile sampling device holder.

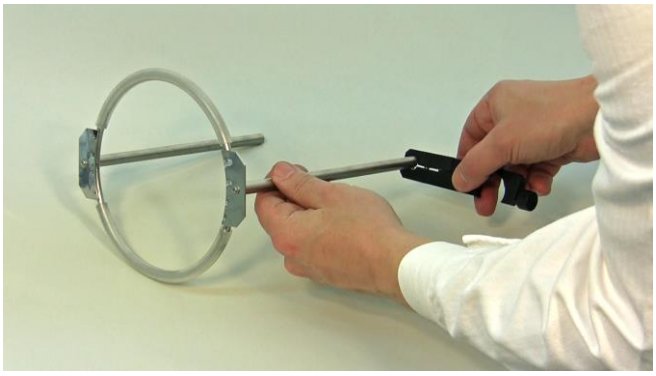


Figure 3.4-2 Insert the holder of sterile sampling device into the reaction vessel holder.

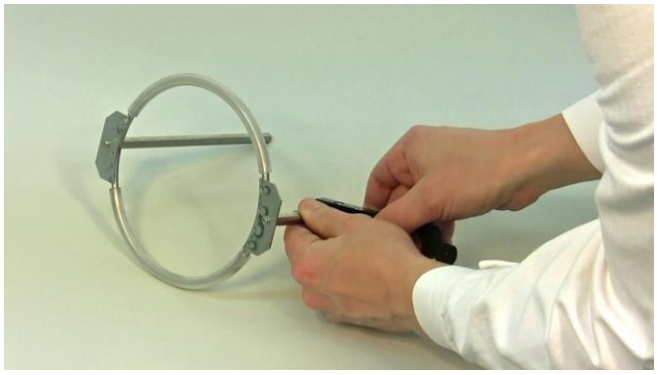


Figure 3.4-3 Position the sterile sampling device holder (approximately near the top) on the reaction vessel holder, so that there is sufficient place for the sampling device and also for the sampling vessel/tubes to be placed underneath.

Figure 3.4-4 After inserting the sterile sampling device holder and positioning it, fix the corresponding screws as shown with the given key to make it hold firmly.

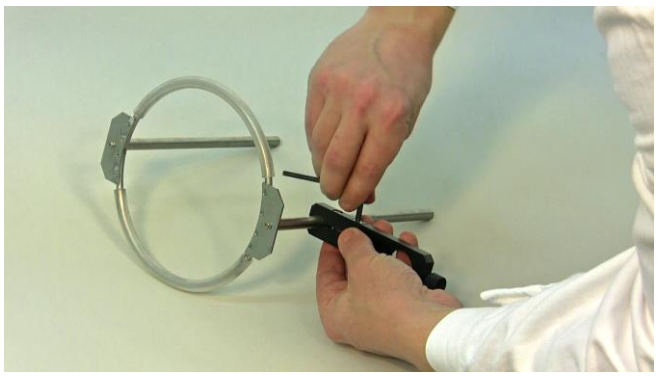


Figure 3.4-5 Also tighten the other screw like before, as shown in the figure. (N.B. If you will need to use the sterile sample device)

Figure 3.4-6 The vessel reaction holder with the sterile sampling device holder needs to be fixed on the MINIFOR base control unit.
Place the reaction vessel holder in the 2 desired slots on either side of the sun-like heating element.

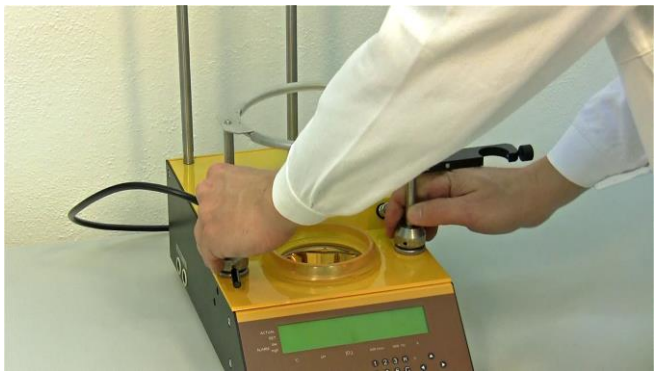


Figure 3.4-7 Deeply insert the reaction vessel holder into the desired slots.

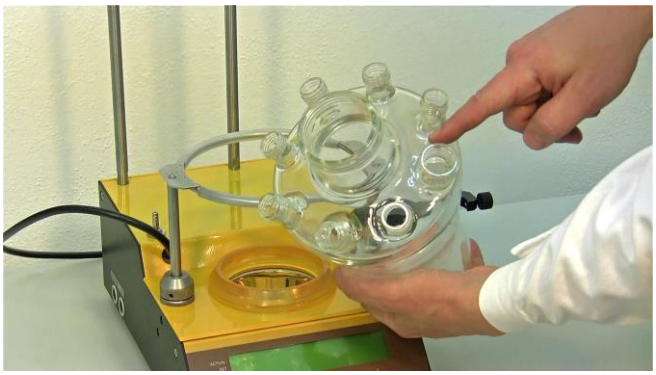


Figure 3.4-8 The port displayed (bigger i.e larger in the diameter port) is the quadruple port for sterile sampling and also for the addition of acid, base.

Figure 3.4-9 The port next to the quadruple port is the port usually defined for pO_2 Probe. The port just opposite to pO_2 probe is the port defined for pH probe. These ports for the probes are specially designed (placed nearly on the circumference of the vessel while the other ports are somewhat placed inside) to avoid the interference with the agitation unit.

Figure 3.4-10 Adjust the deeply inserted reaction vessel holder around the reaction vessel so that it is supported well. This may be a little stiff, so please be careful.

Figure 3.4-11 While supporting the reaction vessel holder with one hand, tighten around the slots of the reaction vessel holder with the given key.

Figure 3.4-12 Tighten the slots also on the other side so that it's secure. (N.B. If you will need to use the sterile sample device you can attach the holder to e.g. the right-hand support)

The above installation guidance resembles the installation of reaction vessel holder for 0.4L, 1L and 3L vessels. The reaction holder for 0.3L and 7L will be different from this type of reaction holder.

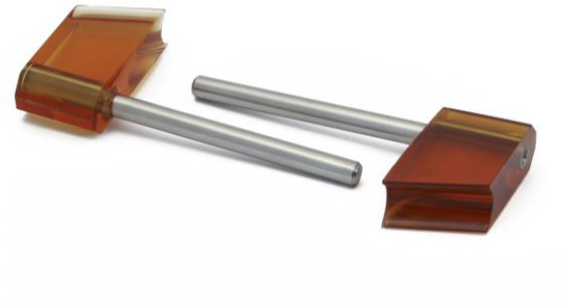


Figure 3.4-13 Adjustable lateral reactor holder for 0.3L reactor.

Figure 3.4-14 Adjustable lateral reactor holder for 7L reactor.

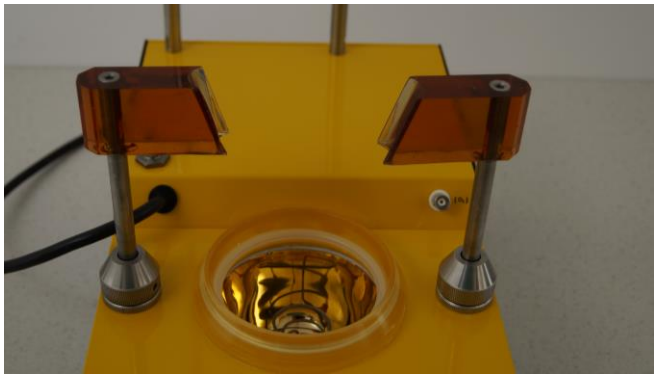


Figure 3.4-15 Insert the adjustable reaction holders on each slot of the MINIFOR base control unit and tighten the slots with the provided key.

Figure 3.4-16 Place the reaction vessel on the base unit with which the distinct side arms for pH and pO_2 probes are supported by the lateral reaction holders. The elastic silicone ring helps to affix the vessel with the reaction holder.

Figure 3.4-17 Place the elastic silicone ring on the distinct side arms of the pH and pO_2 probe.

Figure 3.4-18 Pull the elastic silicone ring and affix the side arms of the vessel with the lateral reaction holder.

Figure 3.4-19 The reaction vessel mounted on the base unit and made intact with the help of the lateral holder and elastic silicone ring.

Never heat an empty reactor! The heat radiation is absorbed by the glass walls and their temperature would increase to such an extent, that thermal dilatation would lead to a vessel burst.

The small jacketed 0.3L reactors must be used only with the vessel jacket **completely filled** with water. The hose connectors of the jacket should be closed (e.g.) with silicone tubing.

The **best range for the automatic temperature control** is between 4°C above ambient temperature and 60°C. Higher temperature you may reach by isolating the vessel with flannel and aluminium foil.

3.4.1 Setting up the 7L reactor vessel

The 7L MINIFOR vessel consists of two glass parts that needs to be fixed together. In the following, the preparation of the liquid O-ring and setting up of the vessel will be explained.

3.4.1.1 Preparation of LAMBDA Liquid O-ring for the 7L MINIFOR vessel

The liquid O-ring consists of a new partially hardening silicone resin filled with the purest quartz powder of extremely fine (fume like) consistency.

Figure 3.4-20 LAMBDA Liquid O-ring ingredients: Solution A, Solution B, Aerosil

Table 7 Preparation of LAMBDA liquid O-ring

	Ingredient	**Preparation**	**Parts** (weight or volume)	**Proposed**
1.	Solution A	Mix solution A with solution B.	1	2 g
	Solution B		1	2 g
2.	AEROSIL powder	Add AEROSIL in such amount until the mixture is no longer free flowing and with a consistency similar to toothpaste is reached		

3.4.2 Set-up of the 7L MINIFOR vessel with liquid O-ring

Figure 3.4-21 Get ready with the reaction vessel, Liquid O-ring ingredients and the closing belt.

Figure 3.4-22 Take equal parts of solution A and solution B in a non-glass beaker. For example: 2g of Solution ‘A’ and 2g of Solution ‘B’. Aerosil powder is added in such an amount until the mixture is no more flowing.

Figure 3.4-23 Transfer the prepared liquid O-ring into a syringe for an easy application of the mixture in the vessel.

Figure 3.4-24 Apply the mixture on the whole circumference of the lower vessel part's brim like a "spaghetti".

Figure 3.4-25 Lower part of the vessel with the liquid O-ring.

Figure 3.4-26 Apply a small amount of silicone oil on the vessel upper part's brim for easy removal of the parts after completing the experiment.

Figure 3.4-27 Place the upper part onto the lower vessel part.
The paste fills any cavity between the joining surfaces and is completely gas tight and also does not generate any stress in the glass.

Figure 3.4-28 Fix them together with the segmented vessel closing belt.
The mixture will cure either during staying at room temperature in several hours or within minutes in the autoclave during sterilization.

The liquid o-ring forms a large surface joint and not just a few mm large contact as it is the case with standard o-rings.

Liquid O-ring can be reused many times. The polymerized silicone joint can be removed manually and cleaned, for example: with alcohol or acetone or it can also just be wiped away with a piece of paper.

3.5 Agitation Unit and Sparger

The next step is to mount the agitation unit along with the air sparger on the reaction vessel for all the sterile applications.

Axis of thread is 60º (do not force)

During setting up the stirring axis, pay attention that you **do not touch the tips of the pH or pO_2 probes**.

Therefore, it is highly recommended to **mount the stirrer axes first** and then the probes.

If you are going to use Minifor without stirring then put **the stirrer set-point to 0** (zero).

With the **optional automation software SIAM**, you can create tools to change the stirrer frequency according the pO_2 value. That may be useful during O_2-limited phases (with maximum aeration) of automatic standard fed-batch.

Figure 3.5-1 Self-cleaning micro sparger along with the assembling parts.

Figure 3.5-2 Sparger has to be assembled with the given parts like shown.

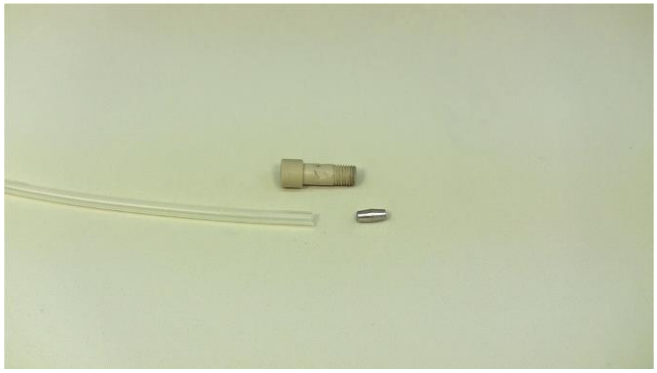


Figure 3.5-3 The items displayed needs to be connected together for LAMBDA PEEk double-seal tubing connector.

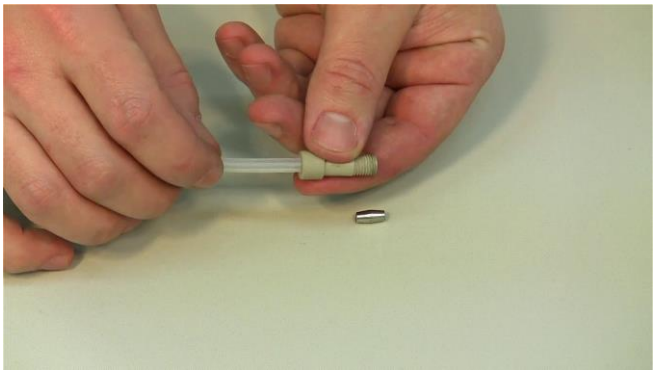


Figure 3.5-4 Insert the tubing into the double-seal PEEK fitting-part.

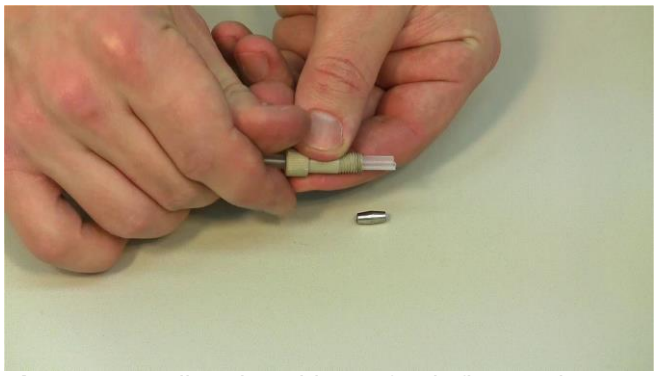


Figure 3.5-5 Allow the tubing to freely flow on the other side of the double-seal PEEK fitting-part.

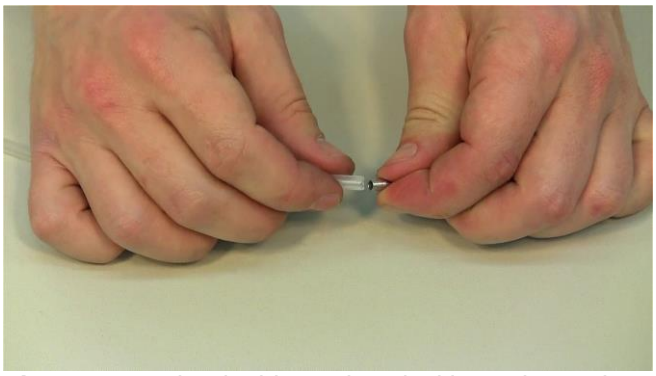


Figure 3.5-6 The double-seal conical insert has to be inserted into the tubing.

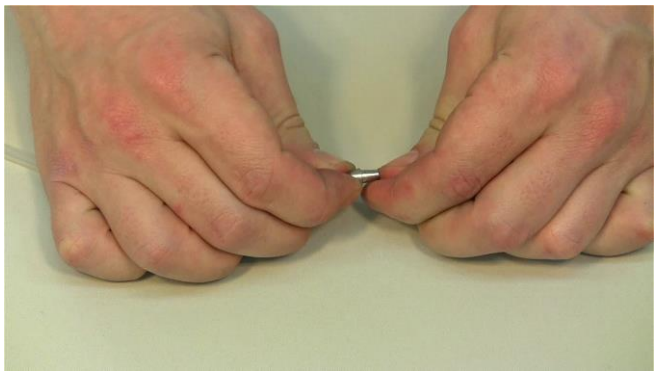


Figure 3.5-7 Insert the double-seal conical insert into the tubing which is freely flowing on the other side of the PEEK fitting.

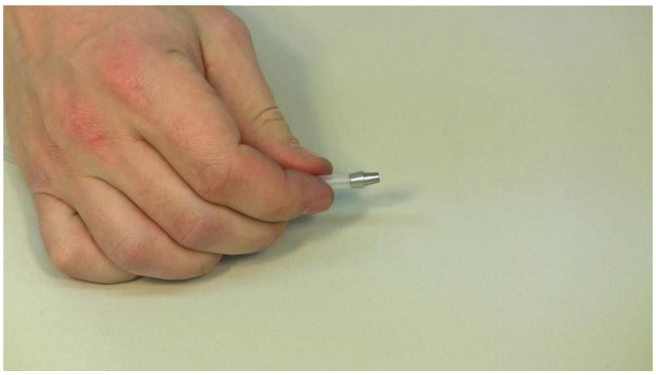


Figure 3.5-8 The resulting double-seal conical insert with the tubing should be like in figure i.e. half the portion of the conical insert can be found inside the tubing.

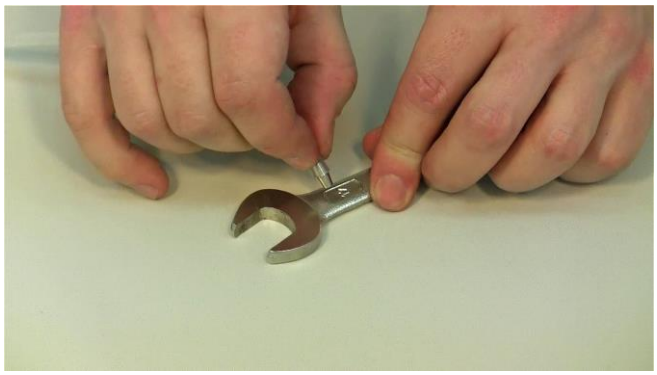


Figure 3.5-9 Take a spanner and the double seal conical insert with the tubing.
Press the conical insert with the tubing against the spanner to make it inserted totally into the tubing.

Figure 3.5-10 Picture shows the prepared PEEK double-seal tubing connector.

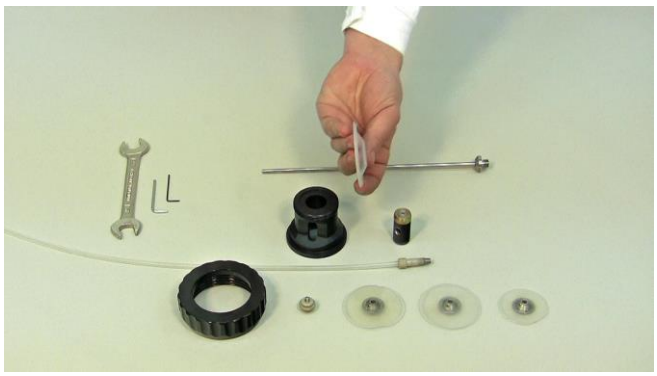


Figure 3.5-11 Displayed is the agitator assembly. The membrane visualized by holding in the hand is the silicone sterility membrane.

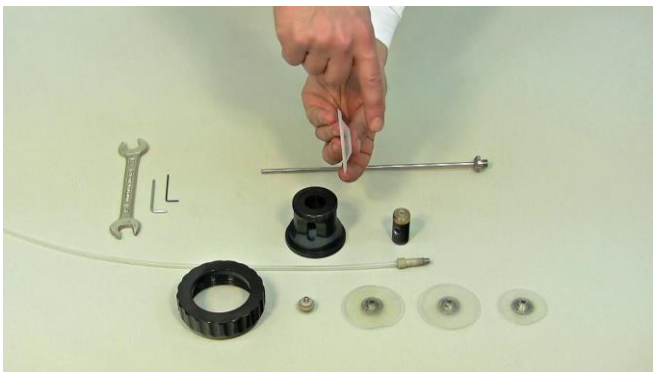


Figure 3.5-12 Hold the silicone sterile membrane so that the convex surface of membrane is outside (as depicted in figure) and concave inside.

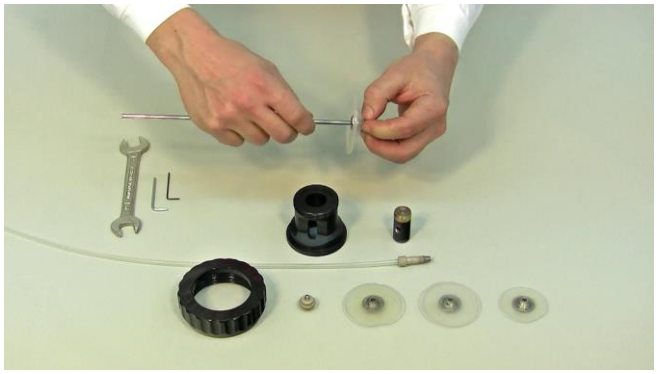


Figure 3.5-13 Insert the sparger pipe on the concave (curved inwards) surface of the sterility membrane. The silicone sterility membrane needs to be fitted properly as shown.

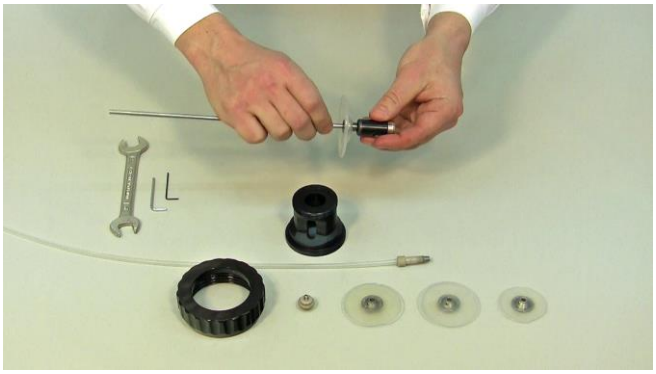


Figure 3.5-14 The mobile cock for magnetic coupling has to be fixed at the threaded end of the sparger pipe. Screw up the mobile cock for magnetic coupling at the threaded end of the sparger pipe.

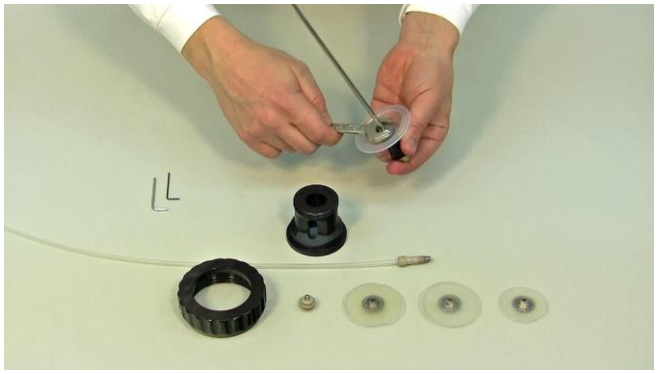


Figure 3.5-15 Securely tighten the nut at the concave side of the silicone sterility membrane which in-turn firmly holds the mobile cock with the membrane.

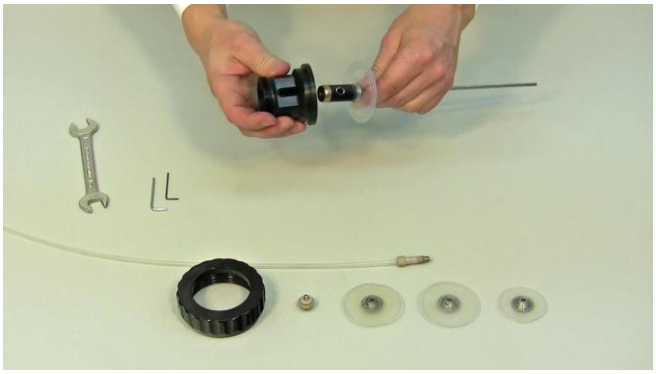


Figure 3.5-16 Place the head of the agitation unit above the prepared mobile cock with silicone sterility membrane in the sparger pipe.

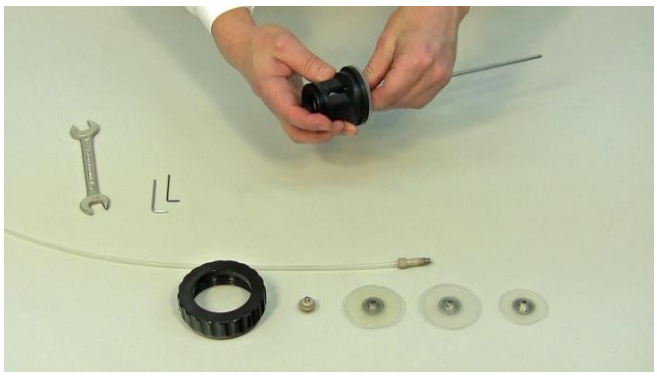


Figure 3.5-17 The head of the agitation unit has to be placed in such a way that the air input tubing holder intended for the double-seal tubing connector is showing through the opening.

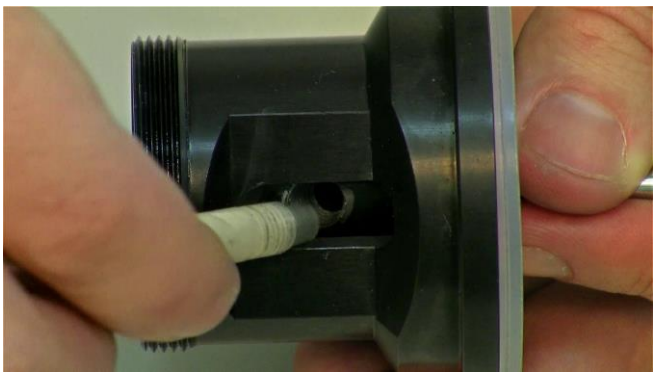


Figure 3.5-18 Insert the prepared double-seal tubing connector into the mobile cock through the head of agitation unit. Screw the double-seal tubing connector clockwise to tighten it.
(ATTN: Axis of thread is 60º (please do not force it))

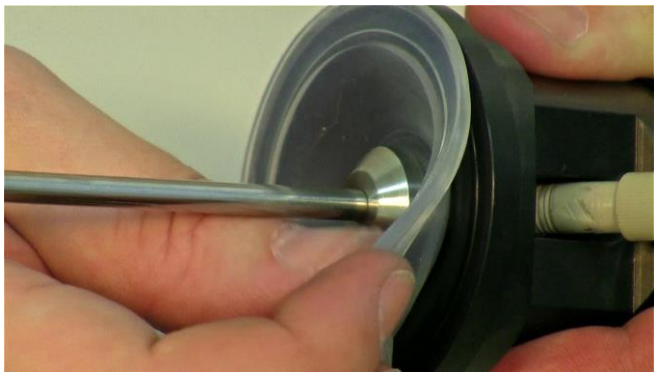


Figure 3.5-19 Ensure that the large silicon sterility membrane is fitted correctly and firmly in the designed grooves on the head of the agitation unit.

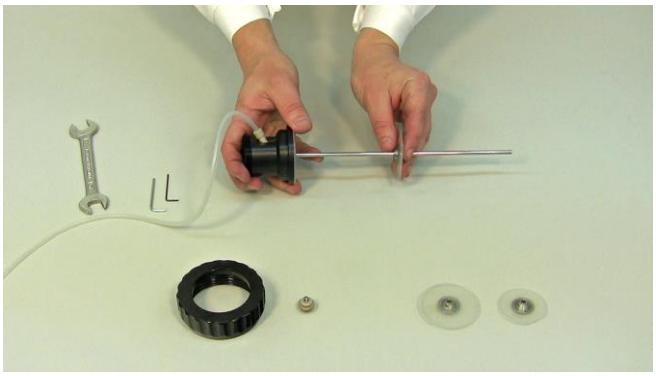


Figure 3.5-20 Start inserting the fish tails in the sparger pipe. This depends on the reaction volume / preferences (usual distance 5-6 cm.
N.B.: can be adapted in number of fish-tails or distance).

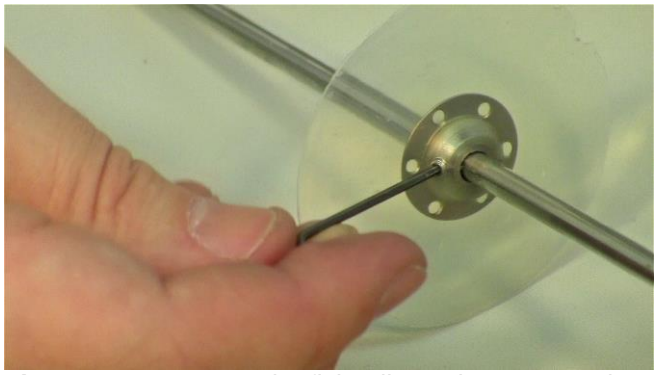


Figure 3.5-21 Fasten the fish tails on the sparger pipe with one of the keys supplied.
(E.g. the screw piece can be facing down for all fish tails)

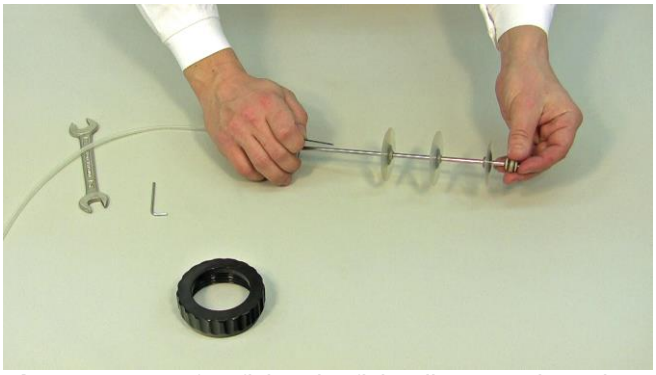


Figure 3.5-22 After fixing the fish tails securely and distributed evenly on the sparger pipe, the self-cleaning micro-sparger has to be added at the end of it.

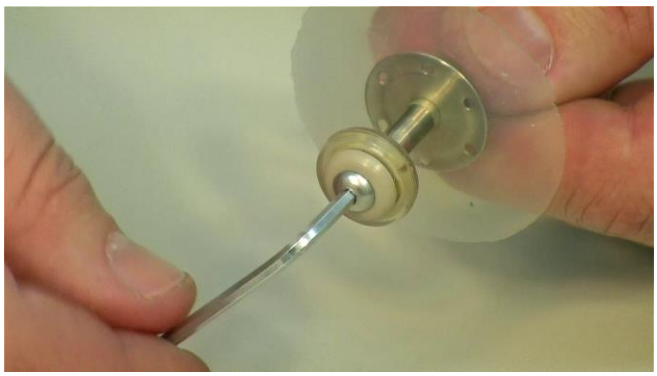


Figure 3.5-23 The key tightening of the self-cleaning microsparger at the end of the sparger pipe.

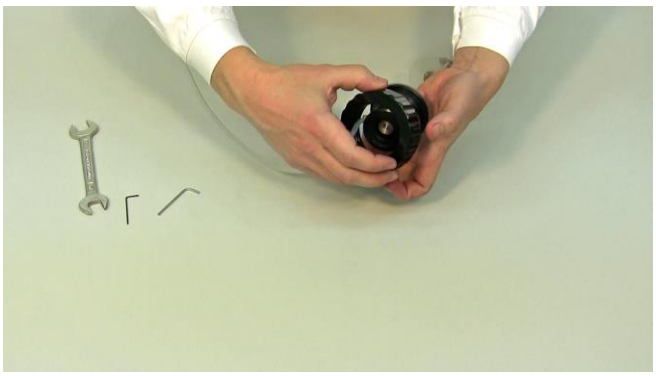


Figure 3.5-24 Place the large central screw cap for head fixing over the head of the agitation unit as shown.

Figure 3.5-25 Place the agitation unit with the fish tails discs and microsparger into the vessel and fix the central screw cap on the reaction vessel.

Figure 3.5-26 Ensure that the large central screw cap for head fixing is securely tightened to the rim of the reaction vessel. See that the mobile PEEK cock tubing for air input is positioned as in the figure.

3.5.1 0.3L Vessel and minimal working volume: Butterfly-shaped stirrer disc – no sparging.

If the 0.3L vessel with minimal volume is used, a special stirrer disk is needed: the butterfly-shaped disk. It allows the insertion of the probes into the minimal working volume.

Figure 3.5-27 Butterfly-shaped stirrer disc for the 0.3L vessels.

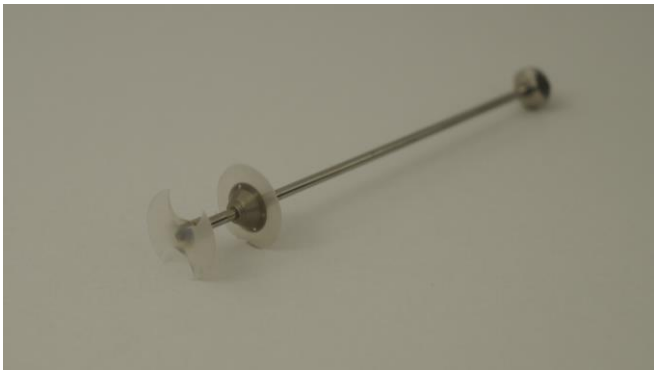


Figure 3.5-28 Butterfly shaped stirrer disc mounted on the sparger pipe instead of the self cleaning microsparger for 0.3L vessel.

If you mount the **butterfly-shaped stirrer disc** instead of the self-cleaning micro-sparger, then do not apply sparging. Therefore **clamp the air tubing between air-inlet-filter and the sparging tube**. See section **3.6.1 Surface aeration**.
Implement a **y-piece** for surface aeration or for sparging through a cannula

Use the MINIFOR **automatic pO_2 control or automatic gas flow control** only with **surface aeration** for minimal volumes in the 0.3L vessels

Do not use the MINIFOR **automatic pO_2 control with sparging** for the **minimal volumes** in the 0.3L vessels.

The internal MASSFLOW delivers at a minimum 100ml gas / min. Therefore, while using minimal working volumes in your 0.3L vessel, replace the sparging by surface aeration.

For the automatic pO_2 control of the minimal working volumes in the 0.3L vessels, you may add an **optional external MASSFLOW 500** and use the optional **SIAM PC-software** “PID controller”.

In case, you would like to apply sparging in the minimal volume of your 0.3L vessel, you **may use a cannula** for it.

3.6 Air-Input

Always use the safety over-pressure valve on the vessel! This will prevent a pressure increase in-case of the blocking of the output filter during excessive foaming. **(Refer chapter 3.9).**
This will ensure that you have the proper filtering from your air input into your fermentation / bioreaction

To make the sliding easy you may **wet the tip of the probe** by **distilled water**.

Figure 3.6-1 Take the free end of the air-input tubing (double seal connector tubing attached with mobile cock) from the head of agitation unit as shown.

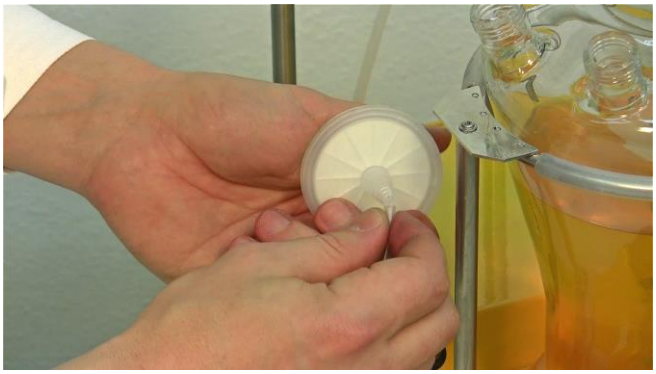


Figure 3.6-2 Connect the air-input tubing from the agitation unit to one end of the air-input filter.

Figure 3.6-3 If the insertion of tubing into the air-inlet filter is found hard to be done. Then use this provided tubing connector for the connection of air-inlet tubing and air-inlet filter.

Figure 3.6-4 Picture shows the connection of the air-inlet filter with the tubing from the head of the agitation unit using the tubing connector.

Figure 3.6-5 Connect a small piece of tubing (cut as desired) to the shown free end of the air-input filter.

Figure 3.6-6 With the other end of the tubing from the air-input filter, connect it to the air outlet on the base control unit as shown.
Insert the tubing deeply till the bottom of the air outlet.

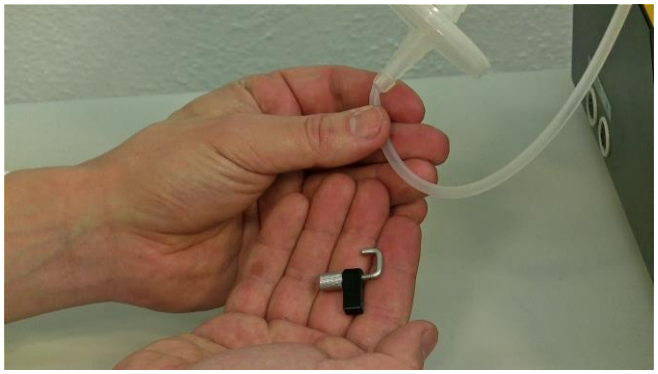


Figure 3.6-7 The safety lock is provided for the air input. This protects the air-input filter for liquids entering the air-output / air-input line).

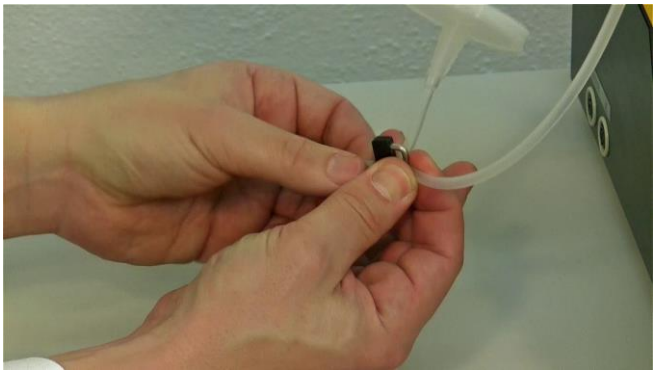


Figure 3.6-8 Secure the safety lock tight on the tubing just before the air-input filter.

(N.B. Cut tubing as desired)

To facilitate the **insertion of tubing**, you can **wet them with a few drops of distilled water**. They will then slide in and out easily.

3.6.1 Surface aeration

The aeration into the headspace (surface aeration) instead of sparging is mainly used for aerobe culturing in **small working volumes** or for any other aerobe culture with a slow consumption of dissolved O_2. It is also possible to use the surface aeration for the diffusion of gases in stem cell culture.

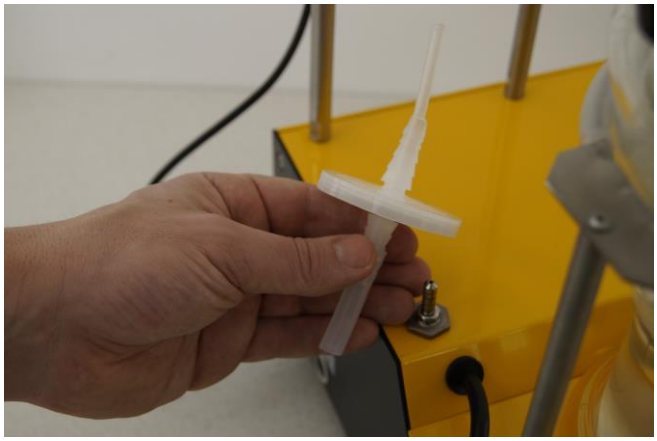


Figure 3.6-9 Insert the tubing with the filter at the air outlet in the MINIFOR base unit.

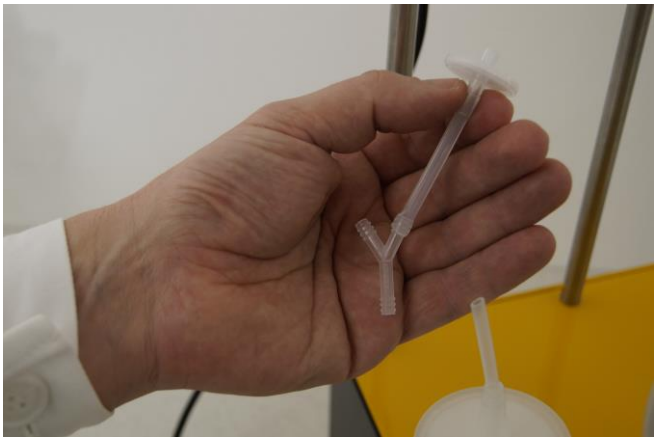


Figure 3.6-10 Add a Y-piece into the aeration tubing.

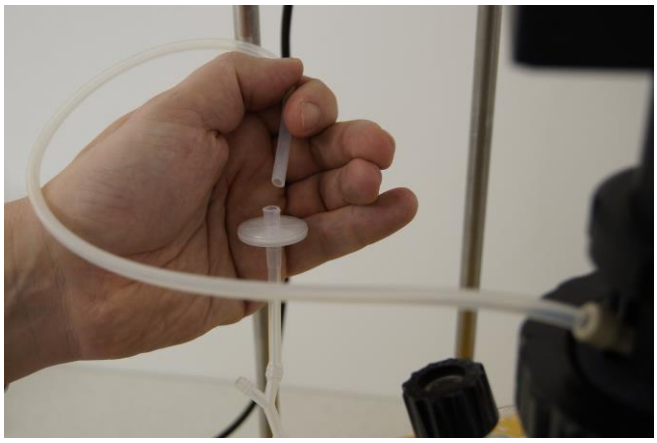


Figure 3.6-11 Connect the tubing with the filter from Y-piece to the sparger.

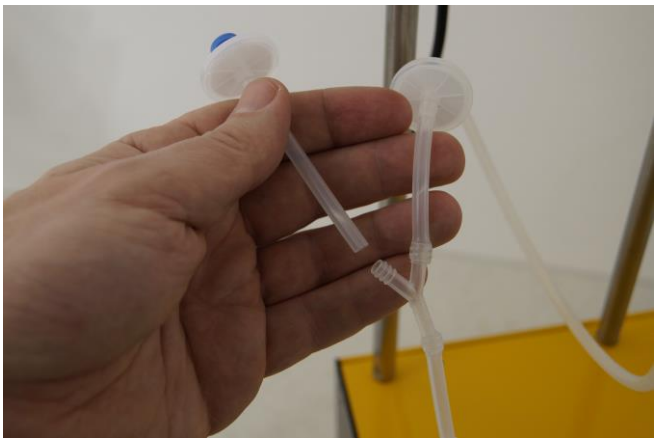


Figure 3.6-12 Insert another tubing with the filter to the Y-piece for surface sparging.

Figure 3.6-13 Connect the tubing for surface aeration to the hollow small cannula in the quadruple sampling port that stays in the head space of the MINIFOR above the medium surface.

Figure 3.6-14 Secure the tubing to the sparger with a lock when surface sparging is used.

3.7 pO_2 probe and setup

The pO_2 probe is included in the MINIFOR Advanced kit or delivered as an option for the MINIFOR Start-up kit.

The pO_2 probe has a slightly larger diameter than the pH probe. It is therefore important to use the **coloured open stopper for the pO_2** probe and any open transparent stopper for the pH probe.

The **female plugs** of cables cannot be cleaned and must be **kept absolutely clean**.

The signal of the pO_2 probe is of very high impedance. Any dirt, salt solution or other **contamination** can negatively **affect the precision** of the measurement.

During the set-up of the stirring axis, pay attention that you **do not touch the tips of the pH or pO_2 probes**.

Do not clean the female plug of the probe cable! (It must be kept absolutely clean)

Never put any cable into the autoclave for sterilization! This is similar for all sterilizable probes.

It is highly recommended to mount the stirrer axis first and then the probes.

Place the tip of the probe about 1 cm from the edge of the closest stirring disc. This will ensure a good exchange of the solution flowing to the membrane.
At the same time it will help to displace air bubbles, which may form accidentally on the membrane.

The contacts of the probes / plugs must be kept **clean**. (Prevent over-boiling at the end of sterilization).

To make the sliding easy you may **wet the tip of the probe with distilled water**.

The contacts on the probe can be cleaned with distilled water and wiped off by clean paper towels.

Measurement of pO_2 during the experiment is easy and can be read in the display. Adjustments, range (minimum and maximum settings), alarm settings, current readings can be controlled and modulated on the MINIFOR Base Control Unit.

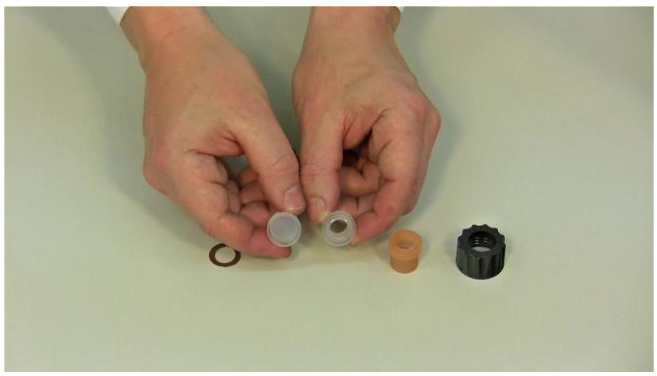


Figure 3.7-1 Before going on with the pO_2 probe installation, ensure the presence of stoppers for blind, the pH and pO_2 (DO) probes (3 types), washers, open screw cap (small) to the reaction vessel.

Figure 3.7-2 Put off the protective cap from the membrane side of the probe. (Protection cap can be removed easily when washed with warm water)

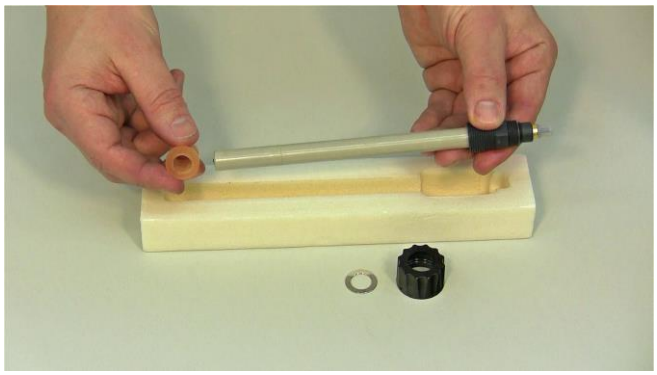


Figure 3.7-3 Use the coloured open stopper for the pO_2 probe as shown.

Figure 3.7-4 Please be careful not to touch this part of the probe.

Figure 3.7-5 Insert the coloured rubber stopper for the pO_2 (DO) probe on the right side of the port which is next to the sampling port (larger in diameter)

Figure 3.7-6 Make sure the washer is placed on to the rubber stopper.

Figure 3.7-7 Place the black screw cap loosely on to the stopper and washer.

Figure 3.7-8 Moisten the pO_2 (DO) probe with de-ionized water to ease insertion of probe.

Figure 3.7-9 Insert the probe through the fixed coloured stopper and screw cap.
Then, push the probe carefully into the port and adjust the height of the probe to be immersed in the medium

Figure 3.7-10 Tightly secure the black screw cap placed on the top of the coloured stopper.

Figure 3.7-11 pO_2 (DO) probe connector have to be connected between the pO_2 probe and the pO_2 (DO) probe socket in the base control unit.
Adjust the sleeve of the pO_2 (DO) probe connector by pushing it up as shown.

Figure 3.7-12 Place and adjust the pO_2 (DO) probe connector by rotating to find the position for exact locking of grooves with the pO_2 (DO) probe.

Figure 3.7-13 After fixing the pO_2 (DO) probe and connector exactly in locking position, push down the sleeve of the pO_2 (DO) probe connector and secure it sufficiently with screw fitting.

Figure 3.7-14 Remove the protection cap by pressing and unlock by rotating from the pO_2 (DO) probe socket on the base control unit.

Figure 3.7-15 Secure the pO_2 (DO) probe connector to the socket by rotating and pressing to lock it together.

To facilitate the **insertion of the probe and the multiple-seal silicone stoppers into the glass necks**, you can **wet them with a few drops of distilled water**. They will then slide in and out easily.

Always place a metal washer under the screw-cap to reduce the necessary screw force!

Do not screw the screw caps with too much force! It is not necessary and the glass threads may break.

3.8 pH and temperature probe setup

The temperature probe is incorporated with the pH probe and it is included in both the start-up and advanced MINIFOR kit.

The pH probe has a slightly smaller diameter than the pO_2 probe. It is therefore important to use the **coloured open stopper for the pO_2 probe** and **any open transparent stopper for the pH probe.**

The female plugs of cables cannot be cleaned and must be kept absolutely clean.

During the setup of the stirring axis, pay attention that you **do not touch the tips of the pH or pO_2 probes.**

Do not clean the female plug of the probe cable! (It must be kept absolutely clean)

Never add any cable into the autoclave for sterilization! This is similar for all sterilizable probes.

It is highly recommended to **mount the stirrer axis first** and then the probes.

The contacts of the probes / plugs must be kept **clean**. (Prevent over-boiling at the end of sterilization).

To make the sliding easy you may **wet the tip of the probe by distilled water**.

The signal of the pH probe is of very high impedance. **Any dirt, salt solution or other contamination can negatively affect the precision of the measurement**.

The contacts on the probe can be cleaned with distilled water and wiped off by clean paper towels.

If a new pH probe is used: Remove the protection cap from the tip of the electrode and rinse the electrode with deionised water. Carefully remove the silicone protection from the diaphragm. If necessary shake the probe gently to bring the solution into the tip part of the glass electrode. Place the probe for at least 24 hours into the buffer solution pH 6-7. (This conditions the glass layer of the electrode and stabilizes the signal.) Before measurements and calibration rinse the probe with distilled water and remove the last drop with filter paper. Wait for the stabilization of both pH and temperature readings.

Refer the leaflet "InPro® 325X, InPro® 325X (ISM), InPro® 325X i" provided along with the pH probe for further information.

Measurement of pH during the experiment is easy and can be read in the display. Adjustments, range (minimum and maximum settings), alarm settings, current readings can be controlled and modulated on the MINIFOR Base Control Unit.

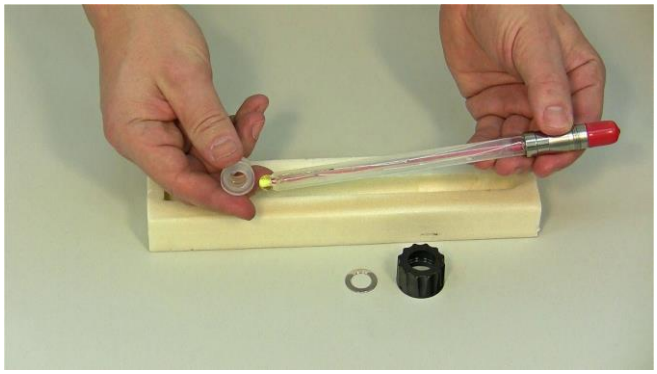


Figure 3.8-1 pH probe can be installed in the ports with any of the white open stoppers provided as shown.

Figure 3.8-2 Care should be taken not to touch the sensor part of the probe.

Figure 3.8-3 Place any one of the provided white open stopper on the port which is opposite to the pO_2 probe port.

Figure 3.8-4 Make sure that the washer is placed on the white open stopper.

Figure 3.8-5 Place the black screw cap loosely on the white open stopper and washer.

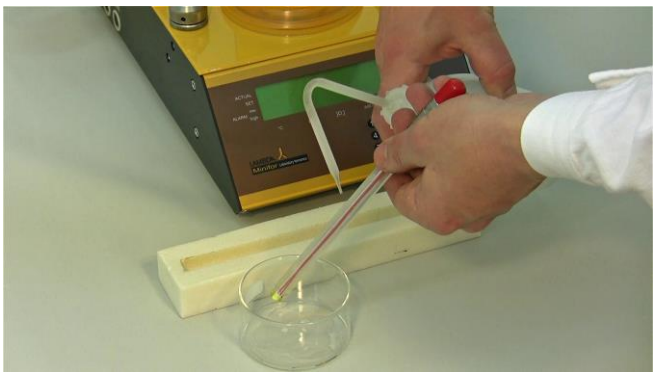


Figure 3.8-6 For smooth insertion of the pH probe on the port, moisten it well with the distilled water.

Figure 3.8-7 Carefully insert the pH probe on the pre-prepared port with the stopper, washer and black screw cap.

Figure 3.8-8 Tightly secure the black screw cap, after insertion of the pH probe.

Figure 3.8-9 Take the pH probe connector from the base control unit of the MINIFOR Bioreactor. (Black cable from the base unit)

Figure 3.8-10 Remove the black protection cap on the sensor (top part) of the cable and red protective cap from the pH probe.

Figure 3.8-11 Connect the pH probe connector (black) cable from the MINIFOR base control unit with the pH probe. Rotate and adjust the connector cable for exact locking of grooves.

Figure 3.8-12 After locking the grooves of the pH probe and connector cable, tighten the connection by screwing the threaded lock on the connector cable by rotating as shown.

Always place a metal washer under the screw-cap to reduce the necessary screw force!

Do not screw the screw caps with too much force! It is not necessary and the glass threads may break.

3.9 Pressure Limiting Security Valve

Always use the safety over-pressure valve on the vessel!

The use of the delivered pressure limiting security valve is imperative! This will prevent a pressure increase in the case of the blocking of the output filter and in the case of excessive foaming.

Maximum input pressure must not exceed 0.2 MPa even for a short period of time! If this happens, the air tubing inside the MINIFOR fermenter will burst and must have to be replaced!

The safety valve must be perfectly cleaned and the **silicone tubing must be replaced after each overpressure event**. Otherwise, the tubing may prevent the correct operation of the over-pressure security valve!

While other systems use rupture discs and get contaminated, the **LAMBDA over-pressure valve prevents contamination** and the run can be usually saved from being contaminated.

Glass is the optimal material for laboratory bioreactors and fermenters. Glass is inert and does not release undesired substances into the medium. Unfortunately, glass is breakable and glass culture vessels do not support high pressures. Under normal conditions there is no problem, because internal pressures in the vessels are low, since the used air (out gas) escapes through the output air filter.

However, when the output filter is blocked (e.g. by too long reuse of the same air filter or by penetration of liquid or foam into the filter) then the real pressure in the vessel can be equal to the gas input pressure*). This may be dangerous with glass vessels. Glass suffers progressively during many sterilization runs and its surface may be scratched accidentally by sand, rings etc. All these effects will lower the final pressure resistance of glass vessels.

*) Recommended input air pressure is 0.1 MPa

When the pressure inside the vessel approaches an overpressure of about 0.1 MPa, the valve starts to leak. As the pressure increases the leak stream is bigger and bigger and the pressure is kept in a safe range. The whistle of escaping air can usually be heard. This should attract the attention of the user that something is wrong. At this moment, the input air pressure must be decreased and the output filter has to be exchanged or a new one needs to be installed.

Overpressure valve assembly and maintenance:

Over-pressure security valve components: Stainless steel cannula with LAMBDA PEEK connector, double-conical tubing insert and overpressure security valve.

The over-pressure safety valve consists of a threaded tube with perpendicular holes covered by a 5 mm long piece of common silicone tubing external diameter 6 mm, internal diameter 4 mm and wall thickness 1 mm. When a critical pressure is attained, the tubing extends and air escapes.

Figure 3.9-1 Insert one end of the double-conical tubing insert to the over pressure security valve.

Figure 3.9-2 Insert the other end with the stainless steel cannula with LAMBDA PEEK connector.

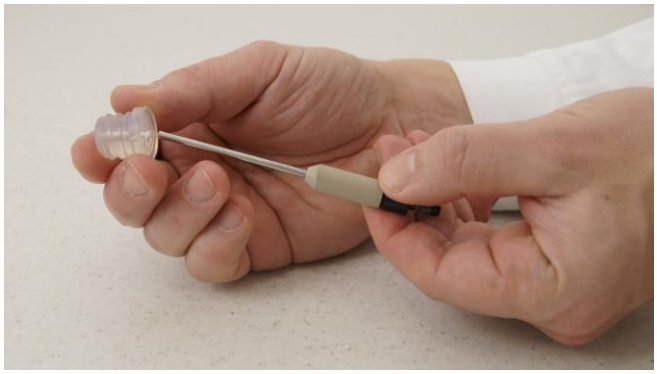


Figure 3.9-3 Insert the assembled over pressure limiting security valve to the rubber stopper.

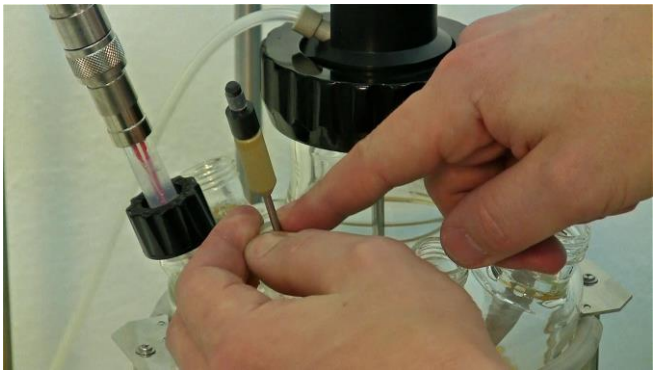


Figure 3.9-4 Insert the stopper with the pressure limiting security valve into the port. (Probably the Pressure limiting security valve can be placed beside the pH probe port on either side)

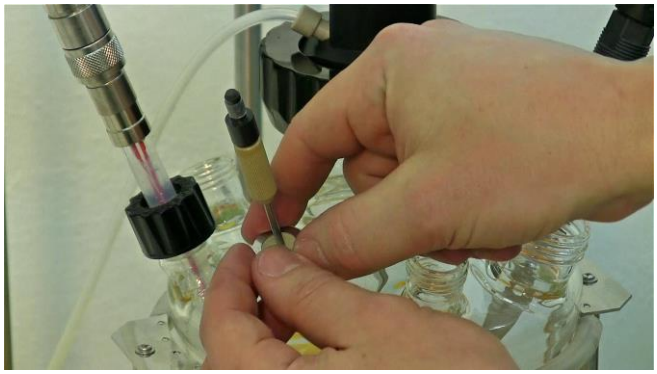


Figure 3.9-5 Ensure that the washer is placed on the stopper with the security valve.

Figure 3.9-6 At last, place the black screw cap and tighten it securely by rotating clockwise.

3.10 Assembly of Sampling and Addition devices

Sampling and addition device includes the sterile sampling device and the quadruple port for taking samples and addition of acid, base or buffers, minerals, nutrients, inoculum into the sterilized medium, etc. respectively.

3.10.1 Quadruple sampling and addition ports

Quadruple sampling and addition ports include the fours cannulas or needles with the with female LAMBDA double-seal connection. The cannulas used can be of stainless steel (Refer Figure 3.10–1) or it can be customized with PTFE tubing instead of stainless steel cannulas (Refer Figure 3.10–2).

Double-seal connection ensures a tight connection and no means of contamination through the ports during the addition of the nutrients, etc and even while sampling.

LAMBDA DOUBLE LOCK connection is made of PEEK. PEEK is a new material similar to PTFE in its extreme chemical resistance and very high melting point (350°C). It can be even flamed. PEEK has a much higher mechanical stability and does not “flow” like PTFE. For its superior qualities, it has been selected by LAMBDA despite of its high price.

In the quadruple sampling and addition port assembly, 3 cannulas with the female LAMBDA double-seal connection are connected with the PEEK double seal tubing connectors that are meant for the addition of acid, base, medium, nutrients, buffers, etc. and sampling. A special septum is used for the cannula that is used for the inoculation.

In the following, the preparation of PEEK double seal tubing connectors, septum for inoculation, sterile sampling device and also the storage bottles that are used for having acid, base, medium, etc. for adding into the working vessel through the port.

To make the sliding easy you **may wet the tip of the tubing and metal parts with distilled water.**

Figure 3.10-1 Quadruple sampling and addition ports with the stainless steel cannulas.

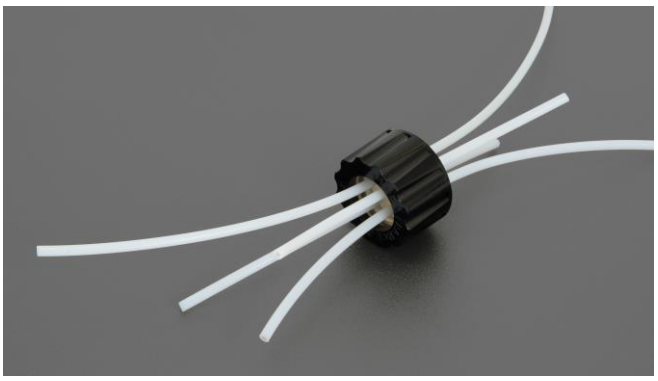


Figure 3.10-2 Quadruple sampling and addition ports customized with PTFE tubing instead of stainless steel cannulas.

3.10.2 Preparation of LAMBDA PEEK double seal tubing connectors

LAMBDA PEEK double-seal tubing connector has to be prepared, like it has been prepared for the installation of agitation unit, to ensure proper closure of the sampling and addition ports.

Now at this stage, the double-seal tubing connectors are prepared for the sterile connection of the respective cannula in the quadruple sampling port with the storage bottle with acid, base, buffers, medium, etc. So, the tubing should have the PEEK double-seal tubing connectors at both the ends of the tubing that needs to be connected with the quadruple sampling port.

To make the **sliding easy** you may **wet the tip of the tubing and metal parts with distilled water.**

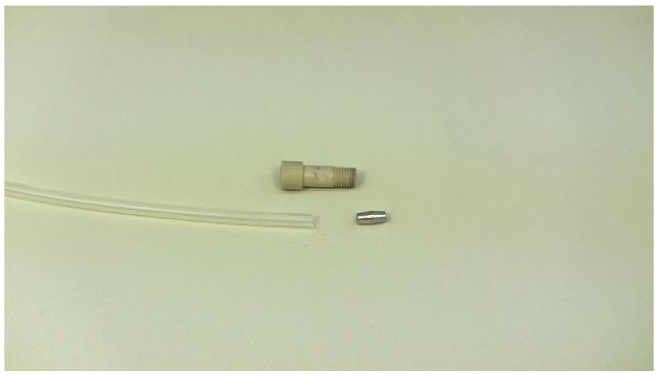


Figure 3.10-3 The items displayed needs to be connected together for LAMBDA PEEk double-seal tubing connector.

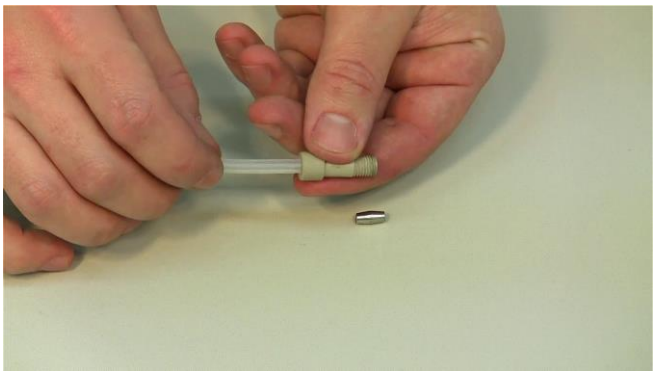


Figure 3.10-4 Insert the tubing into the double-seal PEEK fitting-part.

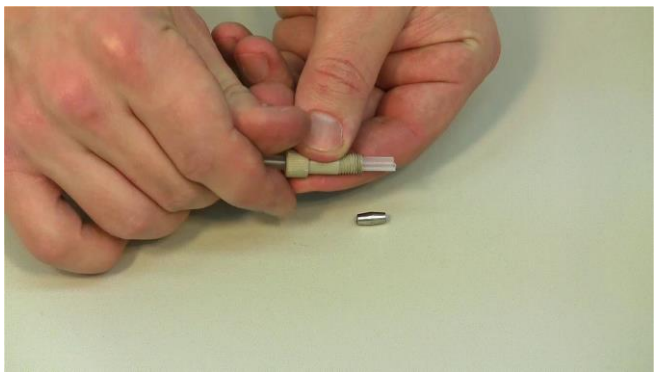


Figure 3.10-5 Allow the tubing to freely flow on the other side of the double-seal PEEK fitting-part.

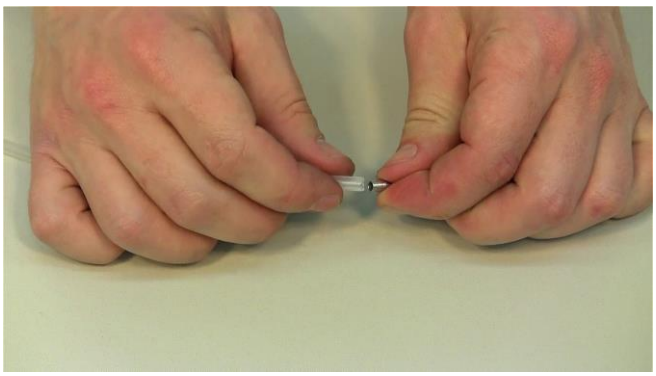


Figure 3.10-6 The double-seal conical insert has to be inserted into the tubing.

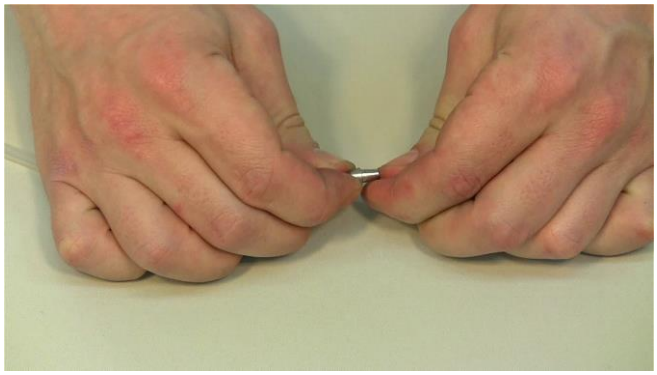


Figure 3.10-7 Insert the double-seal conical insert into the tubing which is freely flowing on the other side of the PEEK fitting.

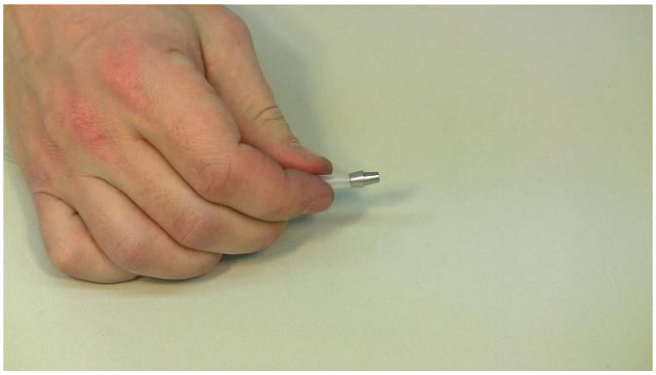


Figure 3.10-8 The resulting double-seal conical insert with the tubing should be like in figure i.e. half the portion of the conical insert can be found inside the tubing.

Figure 3.10-9 Take a spanner and the double seal conical insert with the tubing.
Press the conical insert with the tubing against the spanner to make it inserted totally into the tubing.

Figure 3.10-10 Picture shows the prepared PEEK double-seal tubing connector.

3.10.3 Preparation of Inoculation device (Septum)

Septum is the inoculation device that will be connected with one of the cannulas in the quadruple sampling device.

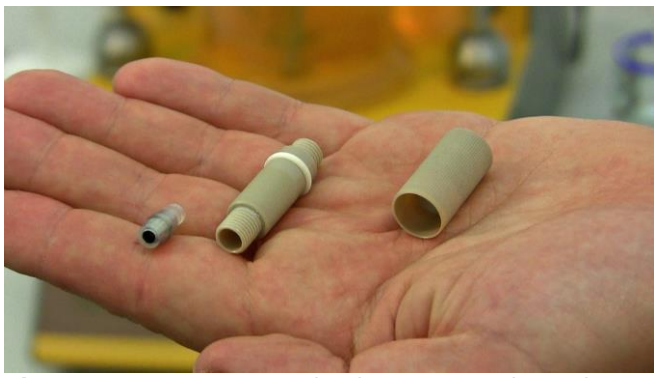


Figure 3.10-11 Parts need to be connected together to form the inoculation device.
From left to right; 1. The tubular septum containing the double seal conical insert for tubing inside;
2. The septum adaptor body with a white septum O-ring for septum adaptor body;
3. The septum protection cap containing the sealing disc.

Figure 3.10-12 The tubular septum (which is sealed at one end) should be fitted over the double seal conical insert with the use of, for example, a hard ruler leaving part of the tubular septum free and exposed as shown.

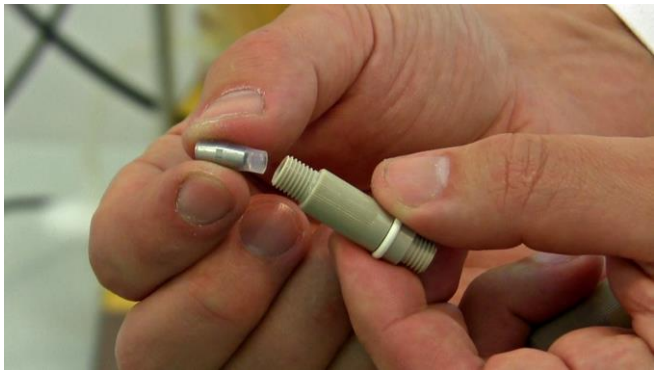


Figure 3.10-13 The prepared double seal conical insert with the tubular septum has to be inserted into the septum adaptor body.

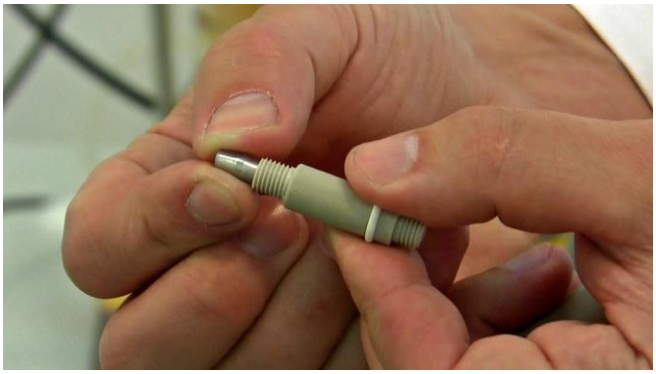


Figure 3.10-14 Note that the sealed end of the tubular septum should be fitted into the septum adaptor body (on the other side of white septum O-ring) such as shown.

Figure 3.10-15 Fix the septum protection cap on the threaded side of the white septum O-ring.

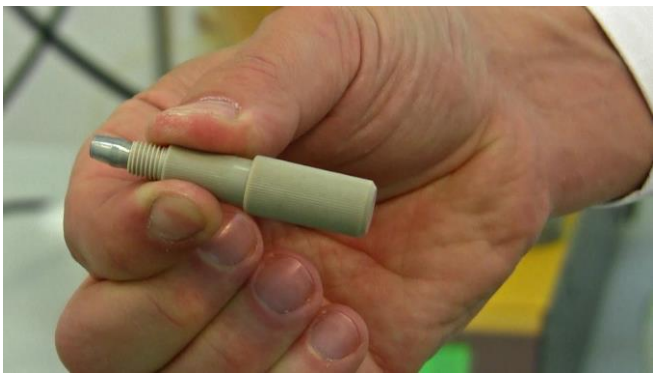


Figure 3.10-16 Complete set for inoculation. The free end with the double seal conical insert has to be inserted on one of the cannulas or needles from the quadruple sampling port.

3.10.4 Preparation of the sampling device

The sampling device is included in each MINIFOR ADVANCED KIT.

Keep the **lines (tubing) between the MINIFOR and the sampling device** as **short** as possible.
(Since the back-flushing is risky for contamination, you will have to do a washing of the line before taking the sample. The shorter the line, the minimum working volume you will lose)

To make the sliding easy you may wet the tip of the tubing and glass parts with distilled water.

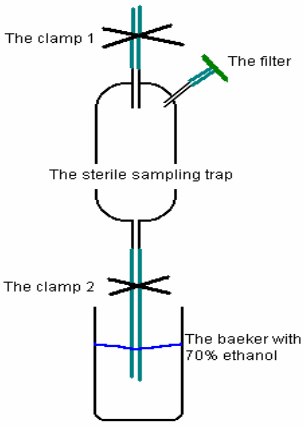


Figure 3.10-17 Schematic representation of LAMBDA Sterile sampling device.

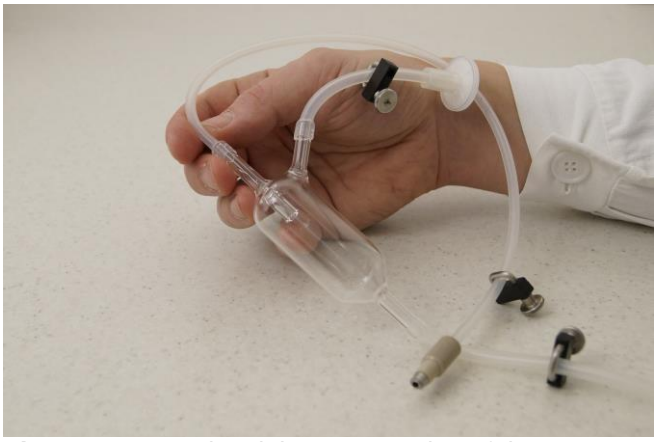


Figure 3.10-18 Pictorial representation of the LAMBDA Sterile Sampling Device.

As depicted in the **Figure 3.10–17 and 3.10–18**, the sterile sampling device is made of glass with 3 provisions for inlet and outlet connections.

- The vertical provision of the glass device which is adjacent to the side provision arm needs to be connected with the Bioreactor for sampling.
- The device has to be connected by the PEEK double seal conical insert tubing with one of the cannulas of the quadruple sampling assembly (the one with the longest needle).
- Clamp the tubing between bioreactor and sampling device before the autoclave!
- A sterilizable air-filter (small ventilation filter 25 mm, 0.2 µm pore size) has to be connected to the side arm by means of short silicone tubing.
- Silicone tubing which is 10 – 20 cm long needs to be connected to the output arm provision on the glass device i.e. the vertical arm opposite to the inlet sampling connection with the Bioreactor (on the lowest part of the device)**.**
- Everything has to be sterilized in autoclave with the vessel.
- Most important is that all tubing must be clamped immediately after sterilization.
- Sampling device has to be fixed in the sampling device holder after sterilization.

Figure 3.10-19 Silicone tubing which is 10 - 20 cm long needs connected to the output arm provision on the glass device i.e. the vertical arm opposite to the inlet sampling connection with the Bioreactor (on the lowest part of the device)

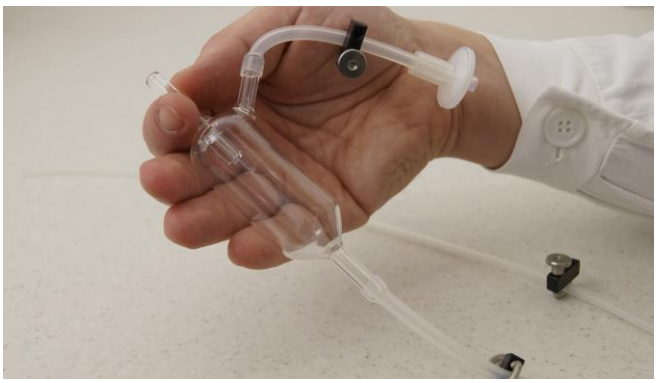


Figure 3.10-20 A sterilizable air-filter (small ventilation filter 25 mm, 0.2 µm pore size) connected to the side arm by means of a short silicone tubing.

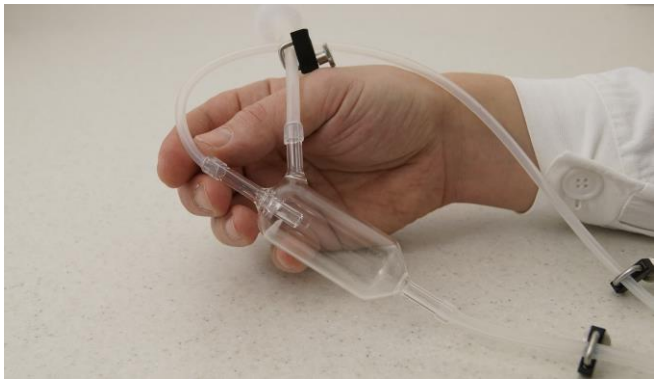


Figure 3.10-21 The tubing with the PEEK double-seal insert needs to be connected with the sterile sampling device. (Refer 3.10.2 Preparation of LAMBDA PEEK double seal connectors) Inlet provision for connecting the PEEK double-seal tubing has a provision tube deep inside the device glass part. (Please refer the picture)

You **may replace the MINIFOR standard sampling device by any other device** (as is standard in your laboratory) with the use of a tubing connection.

3.10.5 Preparation of storage bottles

Storage bottles are the stock bottles used for the addition of acid, base or buffers, medium, nutrients, minerals, etc. by means of automatic pumping into the working vessel.

Figure 3.10-22 Reagent bottle 250ml with pipe (cannula with the female LAMBDA double-seal connection) and Luer-Lock fitting.

Table 8 Storage bottle: ports and purpose

Amount	Needle	Purpose	What has to be installed on it
1	Long	Pumping liquid out of the bottle to the bioreactor (**liquid**)	**LAMBDA double-seal connector** (for preparation, please refer 3.10.2) and tubing leading through the pump on to the quadruple port of the fermentor vessel.
1	Short	Ventilation (**gas**)	**Gas filter** with 0.2µm pore diameter, autoclavable

The filters have to be well fixed on the needle. This may depend on the supplier. If the filter and the needle do not fit, then insert the silicone tubing between the needle and the filter. Fix it with autoclavable cable binders.

Wet tubing can be mounted well on the needles –pass water through the tubing before mounting. Furthermore, the water will be helpful during sterilization since the vapour ensures heat transfer. However, make sure that your filter stays dry.

Do not use HCl as acid for your fermentation and bioreactions as far as your process **allows** others like **H_3PO_4 or H_2SO_4.**

3.10.5.1 Line between storage bottles and MINIFOR vessel

The storage bottles along with the silicone tubing can be sterilized together with the fermentor / bioreactor vessel.

All lines between storage bottles and MINIFOR vessel should be kept as short as possible for easier handling, less contamination and long term usage. You should also take into account the additional length needed for pump head length and/ or welding line.

LAMBDA peristaltic pumps (head) work best with the silicone tubing. Tube welding machine, may need other tubing which is too hard for LAMBDA peristaltic pumps. For the line (between storage bottle and MINIFOR vessel) you can use a different types of tubing which are connected with an autoclavable tubing connector.

In case, autoclavable tubing connectors are used, fix the tubing with a cable binder. (For metal connectors, do not use the cable binder since the silicon tubing will be melted on to the metal after autoclaving).

If you need to know the quantity of liquid added without the use of a balance or if you want to measure the flow rate, then the LAMBDA pump speed / flow rate has to be calibrated.
However, the inner diameter of the silicon tubing may change during sterilization. The first sterilization has the biggest impact on the material. Use only AUTOCLAVED tubing for preparing the lines between storage bottles and MINIFOR vessels.

3.10.6 Mounting of sampling and addition devices

To make the sliding easy you may wet silicon / glass / metal with distilled water.

After the preparation of the quadruple sampling assembly, PEEK double-seal tubing connector, inoculation device and the sterile sampling device, everything have to be mounted in its place in order to make it a complete set-up.

Figure 3.10-23 Insert the quadruple sampling assembly into the port which is larger in diameter and adjacent to pO_2 probe set-up.
Fix and tightly secure it with the black screw cap.

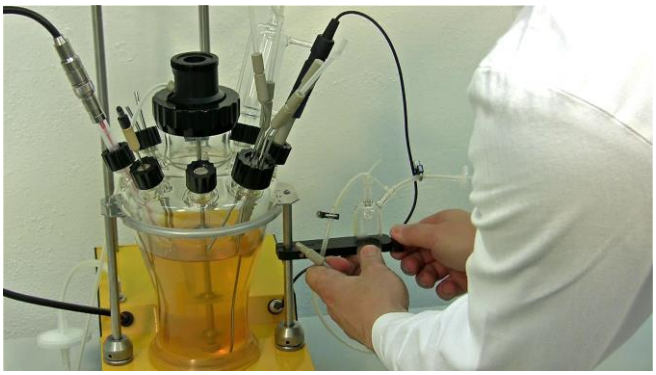


Figure 3.10-24 Place the sterile sampling device on the sterile sampling device holder (Refer 3.4 Setting up of fermentation Vessel) and tighten it with the side-screw.

Figure 3.10-25 Remove the double-seal tubing protection closure on the longest cannula of the quadruple sampling port as shown.

Figure 3.10-26 Take the PEEK double-seal tubing from the sterile sampling device and connect it with the longest cannula on the quadruple sampling port.

Figure 3.10-27 Take the prepared tubing with the PEEK double-seal tubing connectors at both the ends of the tubing (Refer 10.3.2 Preparation of PEEK double-seal tubing connectors).

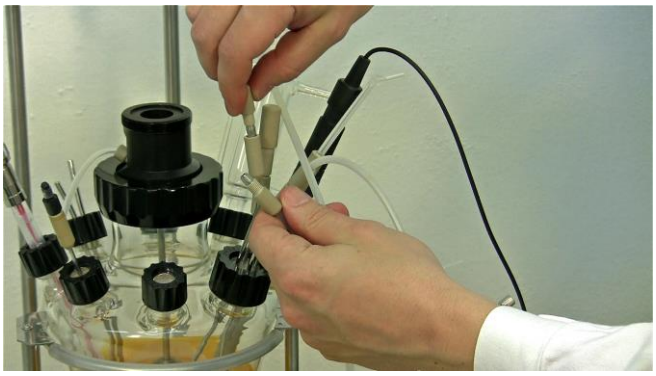


Figure 3.10-28 Connect one end of the prepared tubing with the PEEK double seal tubing connector to the short cannula in the quadruple sampling port.

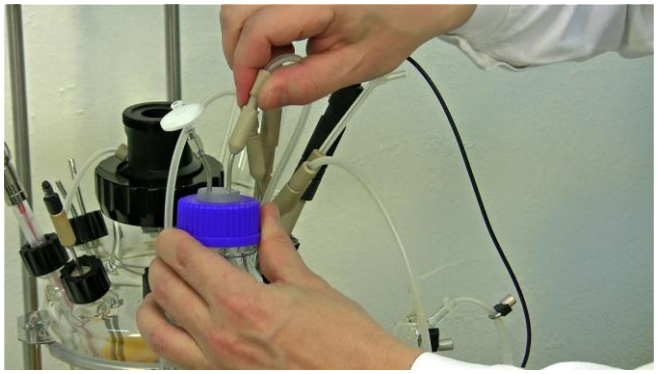


Figure 3.10-29 Connect the other end of the prepared tubing with PEEK double seal tubing connector to the long needle of the storage bottle.

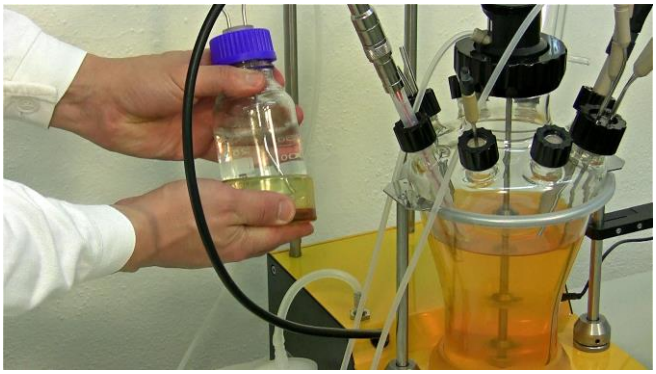


Figure 3.10-30 Stock bottles can be conveniently kept at the back of the MINIFOR base control unit using the magnetic bottle holder.

3.11 Medium cooling device

LAMBDA MINIFOR offers two different ways to cool down the medium:

- Cooling loop: for cooling with cooling liquid (delivered with each MINIFOR KIT)
- Peltier cooling finger based on peltier cell: thermoelectro based cooling, without cooling liquid. (Optional device for up to 3L vessels).

Figure 3.11-1 Cooling loop

Figure 3.11-2 Peltier cooling finger

The cooling system is not connected with the automatic temperature control.
The **automatic temperature control** is **done by the LAMBDA heating system**.

However, **with the cooling**, you should **adjust your warm medium temperature just below the required temperature set-point**. Then the automatic temperature control will achieve the exact set-point for your temperature setting.

Most systems do not need any cooling. (It depends on the working volume, the cell type and density as well as the feed and production phase.).
If you are **not sure whether your system will need cooling**, then **just mount the water cooling loop** as described in the chapter below.
In this way you may **connect the cooling liquid** (tap water or other) as soon as you need it.

3.11.1 Cooling loop

In case the fermentation reaction needs cooling, the cooling loop can be used to maintain the desired conditions.

Each MINIFOR unit is delivered with a Cooling loop for the cooling of medium in the vessel using the cooling liquid.

To make the sliding easy you may **wet the tip of the loop / tubing with distilled water.**

Figure 3.11-3 Place the cooling loop in the desired neck at the back of the vessel to leave it undisturbed.

Figure 3.11-4 Secure and fasten gently with the black screw cap.

This is where the cooling loop should remain until the sterilization of the mounted vessel.

After the sterilization or as soon as there is a requirement for the cooling loop to maintain the working medium temperature, then the connection to the cooling liquid (tap water or liquid from thermostated water bath) has to be made.

Figure 3.11-5 Slip the tubing for cooling liquid inlet over one of the steep tubes of the cooling loop as shown.

Figure 3.11-6 Slip the tubing for the cooling liquid outlet over the other tube of the cooling loop to allow the flow of cooling liquid throughout the cooling loop.

Let the cooling liquid to circulate throughout the cooling loop via the tubing connection for cooling of the working medium.

To facilitate the **insertion of the multiple-seal silicone stoppers into the glass necks** (e.g. probes, cooling loop, cooler, tubing, etc.), you can **wet them with a few drops of distilled water**. They will then slide in and out easily.

Always place a metal washer under the screw-cap to reduce the necessary screw force!

Do not screw the screw caps with too much force! It is not necessary and the glass threads may break.

3.11.2 Electronic Peltier cooling finger

The Peltier cooling finger is an optional thermo-electric device for the MINIFOR kit. It allows the cooling of the working medium without using the cooling liquid and works based on the electronic cooling effect produced by the flow of electric current through a Peltier cell.

The working of the electronic Peltier cooling finger is according to the "heat pipe" principle, which has an advantage of having up to 80 times higher heat conduction compared to copper (!) and can be used with various levels of medium. It works even when the loop is not entirely submerged in the medium.

The LAMBDA Peltier cooling system is extremely compact and advantageous when cultures should be maintained at reduced temperatures. Much lower temperatures can be achieved by isolation of the vessel with convenient isolating material. The LAMBDA Peltier cooling loop eliminates the need of refrigerated circulating baths, which are expensive and takes a lot of valuable bench surface.

The device is **best suited for 1L vessels, maximal 3L vessels.**

Do not install the Peltier medium cooling device on 7L MINIFOR vessels.

Never put the Peltier cooling element or any other electronics or parts with cables **into the autoclave**. This would destroy them!

During Sterilization: the motor and fan unit has to be detached from the cooling pipe and attachment.

Figure 3.11-7 Detach the motor and fan unit from the cooling pipe as shown while preparing for sterilization.

Figure 3.11-8 Detached parts of the Peltier cooling finger.

After sterilization: The Peltier cooling finger has to be prepared by connecting the motor and fan unit with the cooling loop.

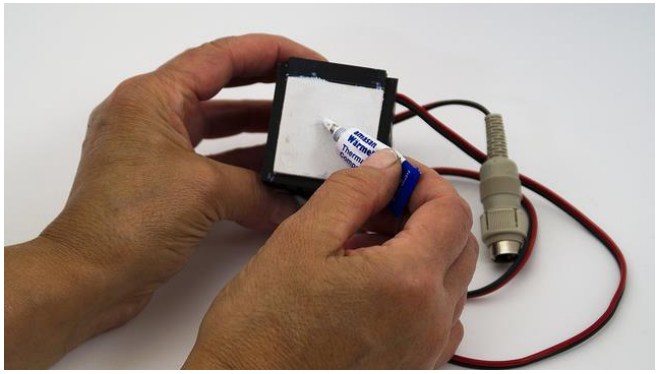


Figure 3.11-9 Apply the heat-conductive paste on the white surface of the Peltier cooling element with control electronics and the fan, before attaching the cooling finger with the unit.

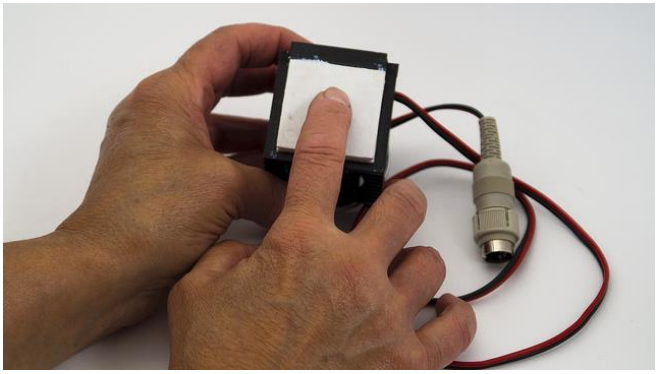


Figure 3.11-10 Spread out the heat-conductive paste on the surface evenly and thinly ensuring that there are no trapped air bubbles.

Figure 3.11-11 Attach the Peltier cooling element with control electronics and fan with the cooling loop by placing the white surface with the heat-conductive paste onto the respective trap in the cooling finger attachment as shown.

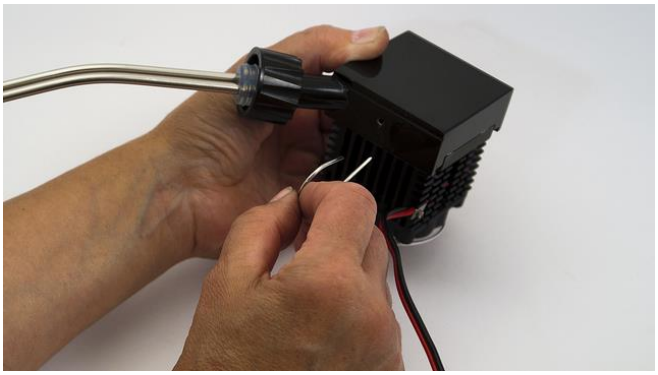


Figure 3.11-12 The Peltier cooling finger needs to be locked using the locking clamp.

Figure 3.11-13 Locking clamp has to be pulled firmly through the Peltier cooling element with control electronics and fan, to ensure good heat transfer to the cooling loop.

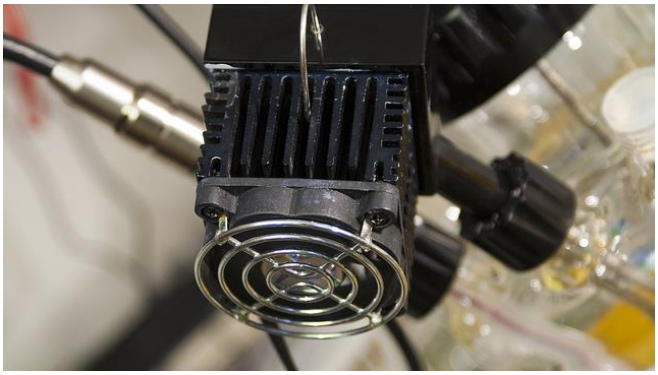


Figure 3.11-14 Peltier cooling finger can be placed in the same place as the cooling loop placed (Refer 3.11.1 Cooling Loop). Secure it tightly with care using the black screw cap, since the head of the Peltier cooling may be slightly heavy while fastening the screw cap.

The connector for the Peltier cooling device has to be plugged into the “PUMP” connector on the rear, right upper corner of the MINIFOR base control unit:

Figure 3.11-15 Connection for the Peltier cooling device: on the rear of the MINIFOR.

For multiple use of the PUMP connector, you can purchase the **quadruple plug box** (see LAMBDA Laboratory price-list).

3.12 Outgas condenser and air outlet filter

The outgas condenser prevents the condensation of water on the output filter and the resulting blocking of the outgas air flow. Condensed water flows back into the vessel.

LAMBDA MINIFOR offers two ways of condensing outgas:

- Glass outgas condenser: for condensing the outgas using cooling liquid (delivered with each MINIFOR kit)
- Electronic Peltier outgas condenser: used without the cooling liquid based on the thermoelectric effect of Peltier cell (Optional)

It is highly recommended to place **an intermediary vessel** between the condenser outlet and the output gas filter. A small amount of convenient **antifoam solution** can be placed on the bottom of this bottle. This will destroy the foam should it enter this additional vessel and will increase the **protection of the output filter against clogging**.

Figure 3.12-1 Intermediary bottle between air condenser and outlet filter
Left side: Air comes into the bottle and leads to the bottom of the flask;
Right side: Air filter mounted on a cannula which leads to the headspace of the bottle

The air outlet needs a sterile **gas filter** (autoclavable, pore size 0.2 µm) to keep the reactor system sterile after sterilization. It is important that the gas filter stays dry and is not affected by condensation otherwise there is a risk of clogging and contamination.

The **outgas condenser** prevents the condensation of water on the output filter and the resulting blocking of the outgas air flow. Condensed water flows back into the vessel.
This flow-back is also important, particularly when working with low volumes for weeks, otherwise the medium would become too concentrated and the working volume would decrease.

To facilitate the **insertion of the multiple-seal silicone stoppers into the glass necks** (e.g. probes, cooling loop, cooler, tubing etc.), you can **wet them with a few drops of distilled water**. They will then slide in and out easily.

Always place a metal washer under the screw-cap to reduce the necessary screw force!

Do not screw the screw caps with too much force! It is not necessary and the glass threads may break.

3.12.1 Glass outgas condenser with air outlet filter

Fix the off-gas-filter with long tubing on the glass condenser. The longest the tubing between the condenser and the filter is better. The tubing has to be maintained in a straight upright position. Care should be taken without forming loops, so that the condensed liquid flows back to the vessel to maintain the constant working volume.

During Sterilization:

Figure 3.12-2 Glass outgas condenser and gas outlet filter need to be sterilized together with the MINIFOR vessel.

Figure 3.12-3 Insert the outgas condenser into the port with the open stopper, washer and black screw cap. Select a port that will not be disturbed during the work.

Figure 3.12-4 Seal the tubing with the “locking strap” as shown. It is recommended to place a small piece of vertically cut tubing above this before sealing with the “locking strap”.

Place a small piece of vertically cut tubing above the outlet tubing fixed on the glass condenser before sealing with the “locking strap”. This would save the tubing from too much of force applied by the locking strap, during sterilization.

After Sterilization:

Figure 3.12-5 Connect the cooling water to the outgas condenser. (Lower arm = inlet, Upper arm = outlet for cooling water).

3.12.2 Assembly of Electronic outgas condenser

The electronic outgas condenser is an optional thermo-electric device for the MINIFOR kit.

It allows the cooling of the outgas without the cooling water and works based on the electronic cooling effect produced by the flow of electric current through a Peltier cell.

The working of the electronic Peltier cooling finger is according to the "heat pipe" principle, which has an advantage of having up to 80 times higher heat conduction compared to copper (!) and can be used with various levels of medium. It works even when the loop is not entirely submerged in the medium.

Never put the Peltier cooling element or any other electronics or parts with cables **into the autoclave**. This would destroy them!

During Sterilization: the motor and fan unit has to be detached from the condensing finger and attachment.

Figure 3.12-6 Detach the Peltier cooling element with control electronics and fan from the condensing finger as shown while preparing for sterilization.

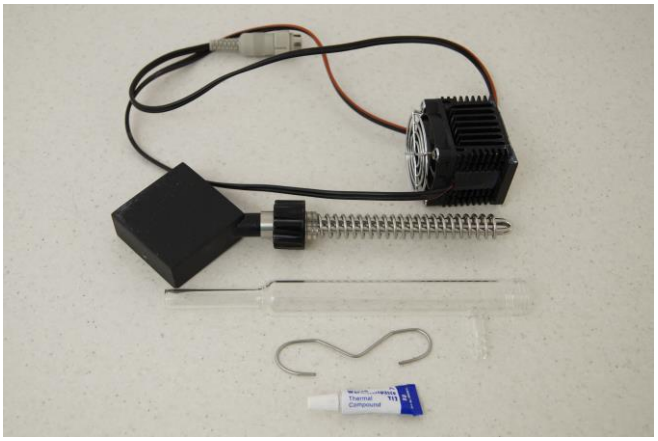


Figure 3.12-7 Detached parts of the Peltier outgas condenser.

After sterilization: The Peltier outgas condenser has to be prepared by connecting the motor and fan unit with the condensing finger.

Figure 3.12-8 Apply the heat-conductive paste on the white surface of the Peltier cooling element with control electronics and fan, before attaching the condensing finger with the unit.
Spread out the heat-conductive paste on the surface evenly and thinly ensuring that there are no trapped air bubbles.

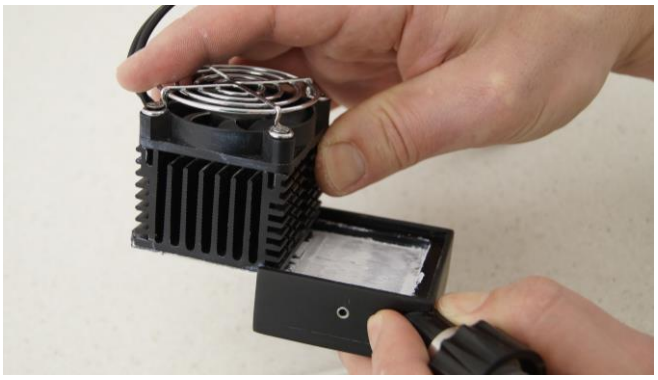


Figure 3.12-9 Attach the Peltier cooling element with control electronics and fan with the condensing finger, by placing the white surface with the thermal paste onto the respective trap in the condensing finger attachment as shown.

Figure 3.12-10 The attached peltier cell with the condensing finger should be made kept together with the help of the locking clamp.

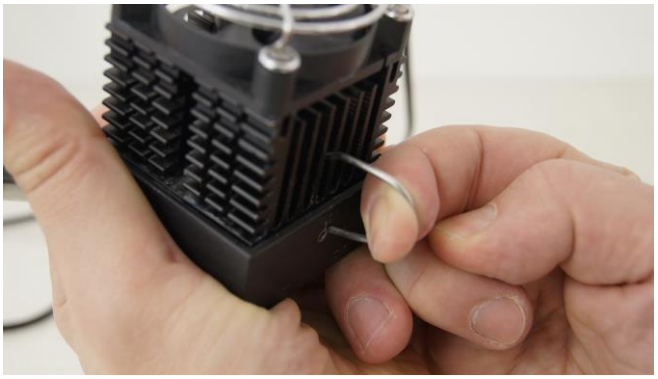


Figure 3.12-11 Locking clamp has to be pulled firmly through the Peltier cooling element with control electronics and fan and fixed into the hole on both sides of the cell, to ensure good heat transfer to the cooling loop.

Figure 3.12-12 Insert the outgas condenser cylinder with the tubing connection over the condensing finger and tighten it securely by the black screw cap.

Figure 3.12-13 Peltier outgas condenser can be placed in the same place as that of glass condenser. Secure it tightly with care using the black screw cap, since the head of the Peltier outgas condenser may be slightly heavier, while fastening the screw cap.

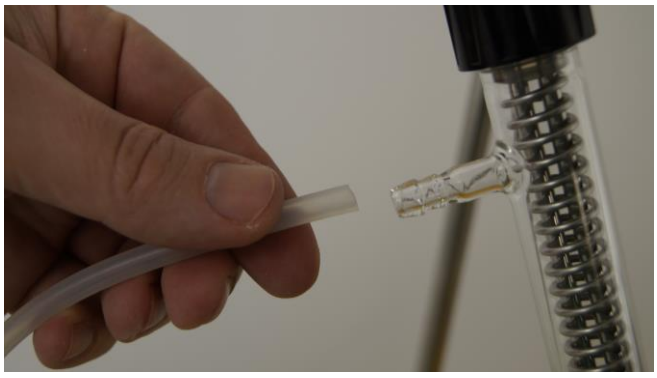


Figure 3.12-14 Connect a tubing to the outgas condenser cylinder and lead the tubing to a glass vessel or flask.

Figure 3.12-15 Connection for the Peltier cooling device: on the rear of the MINIFOR.

3.13 Scale module installation for continuous mode of operation

For the continuous mode of fermentation, the weighing module (chemostat) can be delivered (Optional).

The installation of the scale module takes part at the end of the batch mode of operation and before switching to the continuous mode. Pre-install the system with the weighing module even after sterilization, to be ready in time without any bad impact on the working cell system.

The instrument weight is transmitted through the tip mounted under the front edge of the MINIFOR fermenter base unit. The weight is concentrated on this hard steel tip.

The miniature scale module with all necessary electronic circuits is located in a square tube assuring highest mechanical rigidity.

Figure 3.13—1 The steel hard tip can be found under the front part of the MINIFOR unit (miniature scale module). Place the weighing module under the front part of the MINIFOR.

Figure 3.13—2 The steel tip (miniature scale module) under the front of the MINIFOR unit should be placed in these grooves of the weighing module.

3.13.1 Adapt the measuring range for your working volume / mass

The miniature scale module (a hard steel tip present under the front part of the MINIFOR base control unit) with all necessary electronic circuits is located in a square tube assuring highest mechanical rigidity. There are **several conical holes on the upper surface of the weighing module** allowing the **selection of the optimal measuring weight range** according to the vessel, instrumentation and medium volume used. This range starts from the smallest 300 ml vessel and extends to the completely filled 6L vessel.

When small, light vessels are used select grooves close to the weight sensor (away from the connection cable/support). This will increase the sensitivity of the weight control.

Use the grooves on the other side of the module (close to the cable/support) for heavy **large volume vessels**. Through this selection of the holes, the lever effect between the support and the sensor location (see Figure 3.13-2) is used to increase or decrease the sensitivity of the scale module.
Thereby, the measuring range can easily be adapted.

3.13.2 Connection of the weighing module to the MINIFOR fermenter-bioreactor

Both supply voltage and signal lines are combined in the same cable for the weighing module. Connect the grey cable connector to the socket “X” on the left side of **the** MINIFOR base control unit.

Figure 3.13—3 Connect the weighing module connection cable in the "X" socket beside the “MIXER” on the left side of the MINIFOR base control unit.

Figure 3.13—4 Insert the plug and tighten it securely by screwing the adjustable sleeve present in the plug.

3.13.3 Range selection

Select the range where the measurement or volume control is required:

After the connecting the cable of the weighing module to the MINIFOR base control unit, socket “X” (Refer 3.13.2 Connection of weighing module to the MINIFOR fermentor-bioreactor), the scale module gets activated and the actual weight signal will appear on the display in the first row of parameter “X”.

While selecting the range of the volume control using the scale module, wait until the **colour** of the LED on the **module becomes stable**.

Figure 3.13—5 If the weight of the filled vessel exceeds the measurement range, a roof sign **"^"** will appear in the measured (actual) weight value [Refer Actual value of the parameter "X" in the display] and a **red LED light** will be displayed in the weighing module.

Move the weighing tip of the MINIFOR towards the next groove on the left of the weighing module.
If the sign of overweight **"^"** sign and the red LED light remains still, move the weighing tip again to the next groove towards the left.

Figure 3.13—6 If the vessel weight is too low, an underscore sign **"_"** will appear along with the measured (actual) weight value [Refer Actual value of the parameter "X"] and a **green LED light** will be displayed in the weighing module.

Move the weighing tip of the MINIFOR towards the next groove on the right of the weighing module.
If the sign of the **underweight "_" sign** and green light remains still, move the weighing tip again to the next groove towards the right.

Figure 3.13—7 Repeat the procedures of moving the weighing tip towards the right or left of the weighing module to until the overweight **"^"** sign and the underweight **"_"** sign disappears.

The optimal positioning of the weighing tip on the scale module can be indicated by the yellow light.
(wait until the colour becomes stable before the next adjustment step).

Once the optimal position is obtained i.e. when no over- or underweight range sign appears, the calibration of the weighing module can be started.

For calibration of the weighing module, refer chapter **5.5 X-channel calibration (gauging): Example Weighing module**

3.13.4 Connection of the addition or withdrawal pump

When the culture or fermentation is started by pressing the **R** button on the fermentor control panel, the "PUMP X" will pump away the medium from the vessel, until the preset weight value is attained. In this way, the amount of the culture will be kept constant independently of aeration, stirring intensity and foam formation.

Figure 3.13—8 Connect the corresponding pump to the “PUMP X” *) socket on the rear of fermenter-bioreactor MINIFOR.

Figure 3.13—9 Press the remote button on the connected 'X'-channel Peristaltic pump.

*) The withdrawal pump has been previously connected to the harvesting needle or any other needle reaching into the medium of the quadruple sampling port or a similar device.

3.14 Antifoam control system (ANTIFO & DOZITO) Installation

The automatic antifoam control system is an **optional** tool that can be opted for the automatic foam detection and foam control.

ANTIFO can also be connected to LAMBDA pumps for the automatic addition of antifoam. It may help you whenever you need a bigger amount of antifoam agent to be prepared (continuous mode of big volumes and strong foam formation due to high protein concentration)

3.14.1 Setting up ANTIFO foam detector and controller

Figure 3.14—1 ANTIFO (foam detector and controller).
Place the main body of the ANTIFO on one of the support rods of the MINIFOR laboratory fermenter/bioreactor (e.g. under a pump carrying support). In this way, it will be accessible and occupy minimal space.

Figure 3.14—2 The two crocodile clips of the ANTIFO need to be connected with the cannulas from the quadruple sampling port for the detection of the foam level in the working vessel.

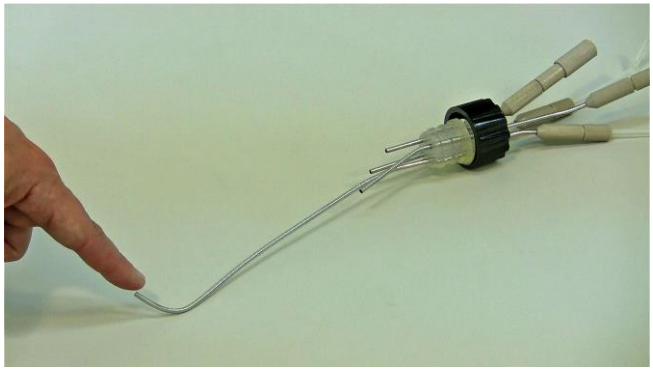


Figure 3.14—3 The harvest needle of the quadruple sampling port (which reaches to the bottom of the vessel) is used as the medium contact.

Figure 3.14—4 Connect one of the crocodile clips to the harvest needle of the quadruple sampling port.

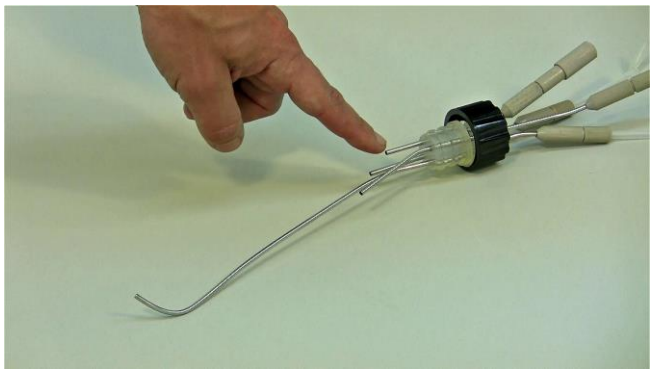


Figure 3.14—5 The second (foam) crocodile clip can be connected to any one of the three shorter needles of the quadruple sampling port.

Figure 3.14—6 Adjust the tip position of the “foam”-needle just above the medium level, where the foam should be controlled. Connect the second crocodile clip to the selected short needle.

For the adjustment of the sensitivity of ANTIFO, refer chapter 5.6.1 Sensitivity adjustment in ANTIFO.

The polarity of these contacts on the cannulas does not play any role, because the measurement is done by a very low and **safe AC voltage**.

3.14.2 Preparation of DOZITO, the Miniature Syringe pump

3.14.2.1 Setting up of Syringe and the anti-foam addition line

A special sterilizable 5 ml glass syringe is used for the addition of anti-foam agent. A PTFE (Teflon) tubing is fitted with a silicone insert and pushed through the syringe connector and tightly screwed on the threaded tip of the syringe.

PTFE tubing, anti-foam addition line, guided through the double conical silicone insert and double seal PEEK fitting part to connect the syringe with the anti-foam with the anti-foam addition port.

Cut approx. 45 cm of PTFE tubing with external diameter of 1.8 mm (of your preferred wall thickness). *(Avoid too long tubing or tubing with a small internal diameter, if you use viscous liquids).*

In case, you just want to refill the syringe, you have to make sure that:

Never fill the syringe when fitted with DOZITO!

Never move the rod which pushes on the piston in the opposite direction!

The pusher rod is pushed out of the pump in the indicated direction only (see above). **You have to remove the syringe for filling!**

To avoid bending the PTFE tubing, introduce a small segment (2-3 cm) of silicone tubing into the threaded connector.

Figure 3.14—7 Push the plunger completely along the barrel of the syringe to mechanically fix the metal outlet tip. Insert the PEEK adapter body on the threaded syringe tip and tighten it securely by screwing.

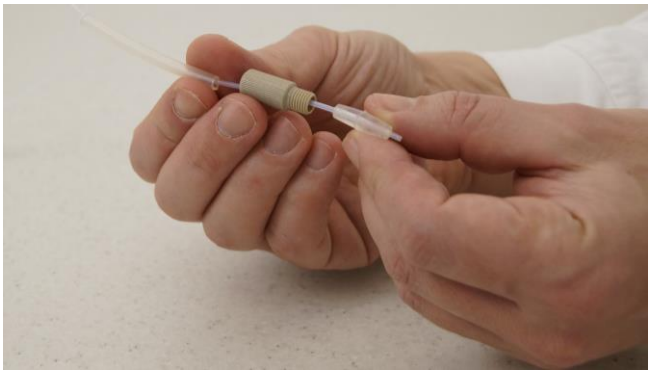


Figure 3.14—8 Insert the double conical silicone insert with the PTFE tubing into the double seal PEEK fitting part through a small segment of silicone tubing, to avoid bending of PTFE tubing.

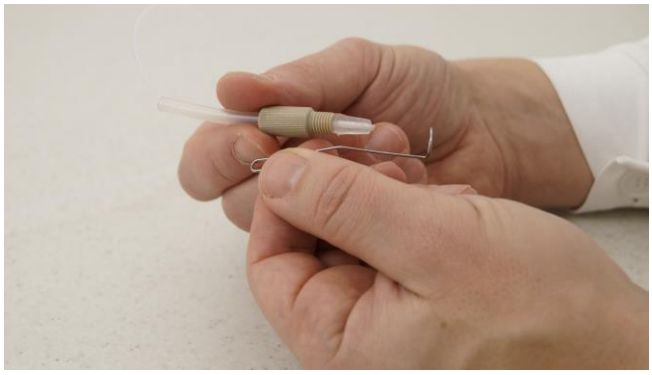


Figure 3.14—9 Insert the holder for autoclaving to the threaded neck of the double seal PEEK fitting part.

Figure 3.14—10 Insert the prepared double seal PEEK fitting part with conical silicone insert and PTFE tubing to the PEEK adapter connected with the syringe.

Figure 3.14—11 Prepared sterilizable syringe with the antifoam line.

Fill the syringe with the antifoam solution.

Viscous liquids are preferably filled by removing the plunger, pouring the liquid into the syringe and re-inserting the plunger in the usual way with the **elimination of air** in the vertical position)

It is preferable to fill the whole length of tubing with antifoam liquid.

3.14.2.2 Preparation of syringe and anti-foam line for sterilization

Never add the ANTIFO and DOZITO syringe pump in the autoclave for sterilization! It will damage them. Sterilize only the syringe filled with Anti-foam.

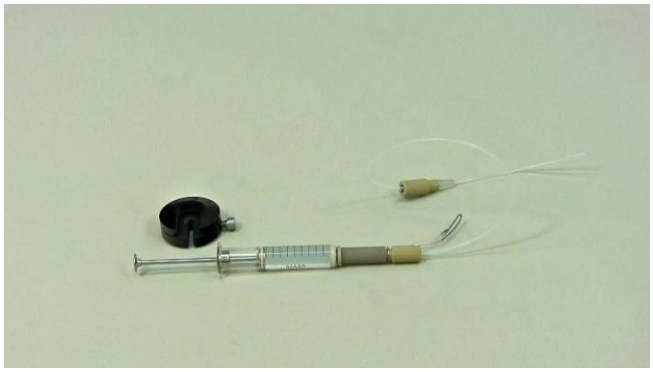


Figure 3.14—12 Take the prepared syringe with the anti-foam and the PTFE anti-foam line with the holder for sterilization.

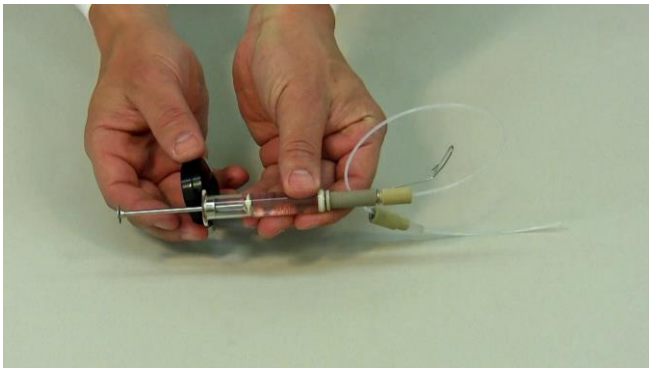


Figure 3.14—13 Insert the syringe bottom to the black syringe holder.

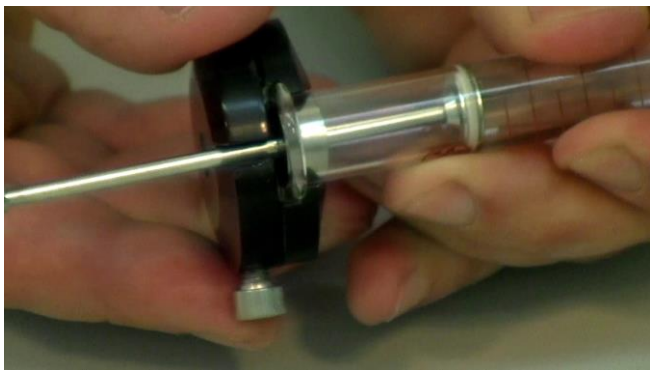


Figure 3.14—14 Rotate the holder with firmly holding the syringe, in order to fit the syringe securely in position.

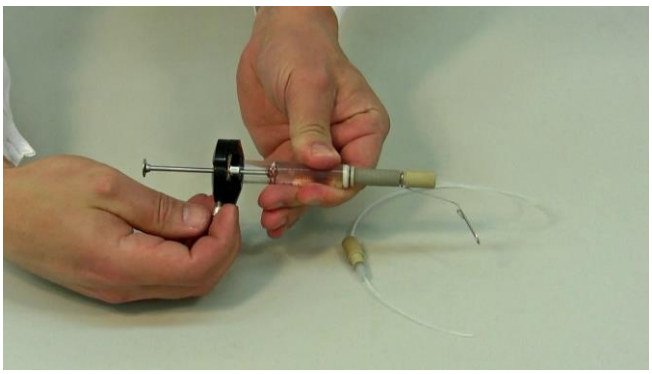


Figure 3.14—15 Tighten the fitted syringe using the side adjustment screw in the black syringe holder.

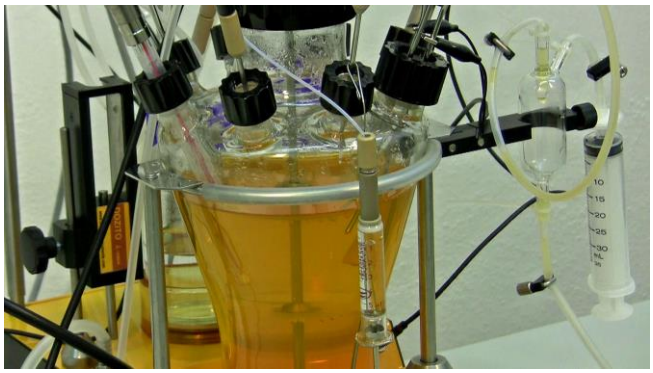


Figure 3.14—16 Hook the Syringe, with the help of the holder fitted in the PEEK head, in the one of the needle/cannulas inserted in the port.

3.14.2.3 After Sterilization: Fixing syringe into the DOZITO Pump

After sterilization, the syringe pump has to be removed from the black holder and need to be inserted into DOZITO (miniature syringe pump).

Never pull the pusher rod in the DOZTIO pump in the opposite direction!

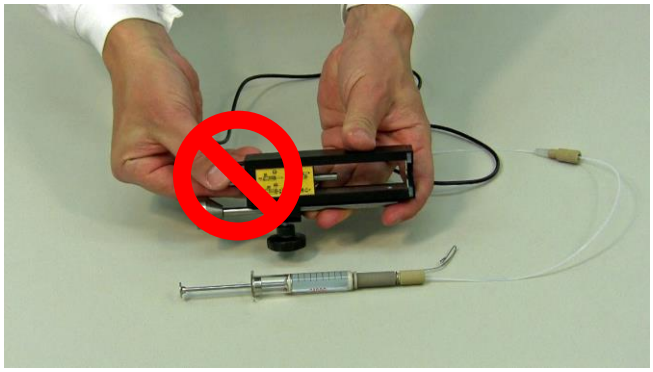


Figure 3.14—17 Care should be taken not to pull the steel pusher rod out of the DOZITO set-up. It may damage the DOZITO Pump.

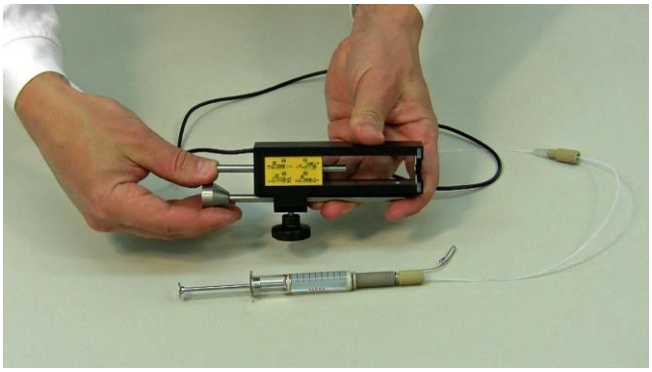


Figure 3.14—18 Always push the steel pusher rod through the DOZITO apparatus to remove it.

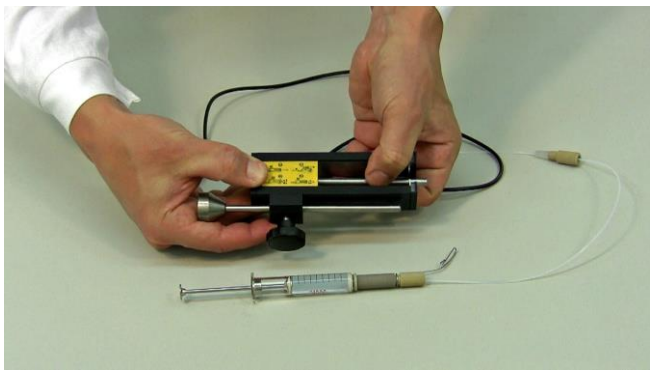


Figure 3.14—19 After pushing the rod totally to the upper part of DOZITO, hold the lower of the DOZITO and pull the pusher rod from above, as shown.

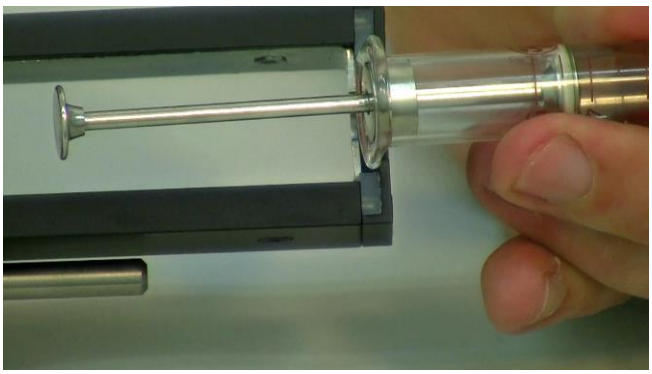


Figure 3.14—20 Place the bottom of the syringe into the syringe pump fixing slot on the DOZITO pump as shown.
Rotate the DOZITO pump by holding the syringe firmly, to make the syringe fit on its position.

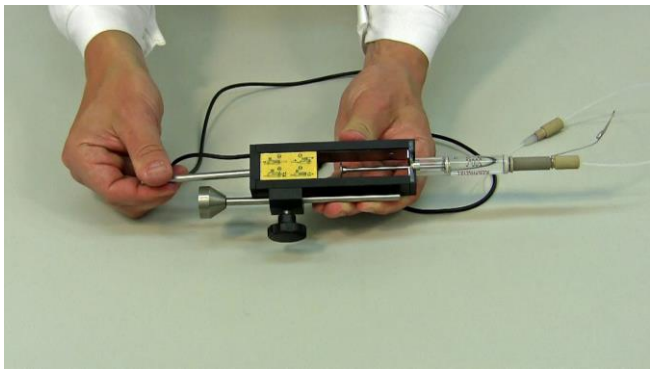


Figure 3.14—21 Introduce the pusher rod through the DOZITO pump.

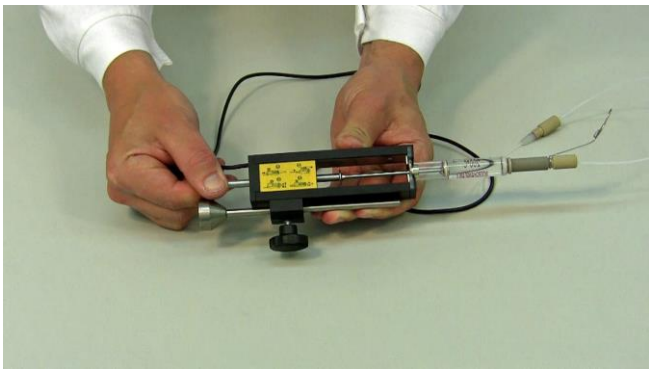


Figure 3.14—22 The plunger of the anti-foam syringe is pushed with the pusher rod until the antifoam liquid just comes out the PTFE tubing tip.

3.14.3 Connecting ANTIFO and DOZITO

The DOZITO, miniature syringe pump needs to be placed on the MINIFOR base unit and connected with the ANTIFO.

Figure 3.14—23 Place the DOZITO on the MINIFOR base unit with the help of the magnetic stand in DOZITO.

F**igure 3.14—24** Plug in the DOZITO's black cable to the black cable of ANTIFO, as shown.

Figure 3.14—25 Connect the ANTIFO cable (8 poles) to the “pump” socket present on the rear of the MINIFOR base control unit.

Figure 3.14—26 The PTFE antifoam line has to be connected with the shortest cannula that will facilitate the addition of antifoam above the liquid level.

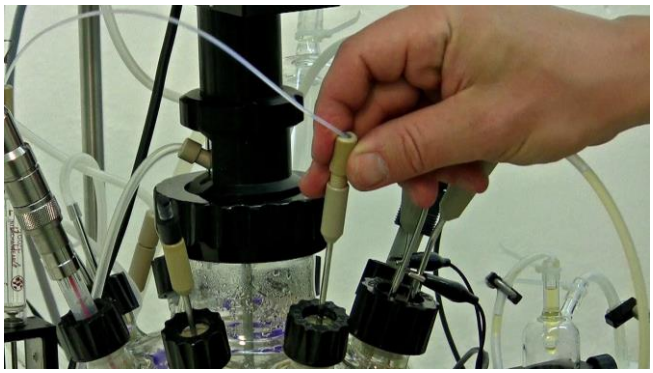


Figure 3.14—27 Insert the PEEK connector with the PTFE Antifoam tubing into the cannula as shown and secure the tubing firmly to the cannula.

3.15 Cable Connections

Everything is clearly labelled to prevent the confusion during cable connections. All connections have their designated sockets with labels.

Never autoclave any cables or electric devices!

Use only the **12V DC** or the remote connection for the pump, MASSFLOW and other additional MINIFOR tools.

3.15.1 Overview of all MINIFOR connections

Table 9 MINIFOR cable connections

	PARAMETER	DEVICE		Socket / inlet on MINIFOR base unit				Other sockets / Inlets
				Front, upper right	Front, upper left	Left	Rear	
1.1	**Agitation unit**	**8 pole cable from the Vibromixer**				“Mixer” socket		
2.1	**Temperature**	Sensor	**Pt 100 incorporated with the pH probe**		pH probe (black) cable			

2.2		Controlled tool	**IR heater (infra-red)**					Internal, connected / ready to use
2.3		Additional tool for the change in temperature range (low)	**Peltier cooling finger for medium**				"PUMP" socket	
3.1	**pH**	Sensor	**pH probe, Mettler**		pH probe (black) cable			
3.2		Controlled tool for acid	**Pump or MASSLFOW**				"ACID" socket	
3.3		Controlled tool for base	**Pump**				"BASE" socket	
4.1	**gas flow**	Outlet gas cooling	**Peltier off-gas cooling device**				“PUMP” socket	
4.2		Inlet gas	**pressured air,** < 0.2 MPa				"AIR" tubing connector	
5.1	**pO_2**							
5.2		Sensor	**pO_2 electrode = DO probe**	pO_2 probe cable				
6.1	**X**	Sensor	**Weighing module**			"X" socket		
6.2		Controlled tool	**Feed or harvest LAMBDA Peristaltic Pump**				"PUMP X" socket	
6.3		Additional tool, not controlled by “X”	**Feed or harvest LAMBDA Peristaltic Pump**				"PUMP" socket	
7.1	**Foam level**	Sensor	**2 crocodile clamps**					Long and short cannula of the quadruple sampling port
7.2		Controlled tool	**DOZITO**					Plug into the black cable of ANTIFO
7.3		Controller	**ANTIFO**				"PUMP" socket	
8	**Grounding**	**Crocodile cable**					"GND"	Crocodile clamp to the long cannula of quadruple sampling port
9	**PC**	**Cable and converter**					"PC" socket	
10	**Alarm**	**Internal and External**					"ALARM" for external	Microproces sor internal
11		**MINIFOR Main power supply**					Power inlet (100 - 240 V, 50 - 60 Hz, max. 1000 W)	

3.15.2 Agitation Unit

The magnetic coupling of the vibromixer with the agitation unit makes the assembly very much handy. Dismantling and assembling the vibromixer with the agitation unit can be done without any difficulty.

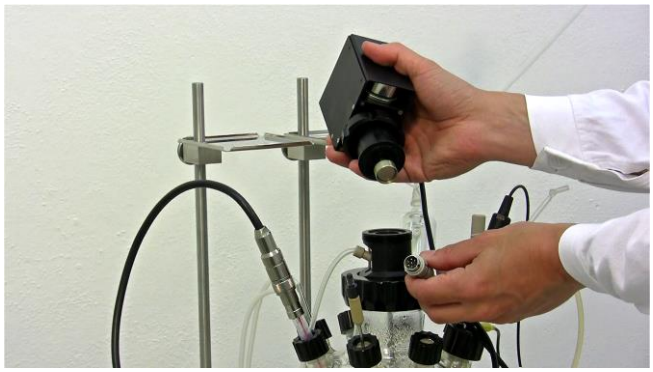


Figure 3.15—1 Take the vibromixer with the cable and fittings.

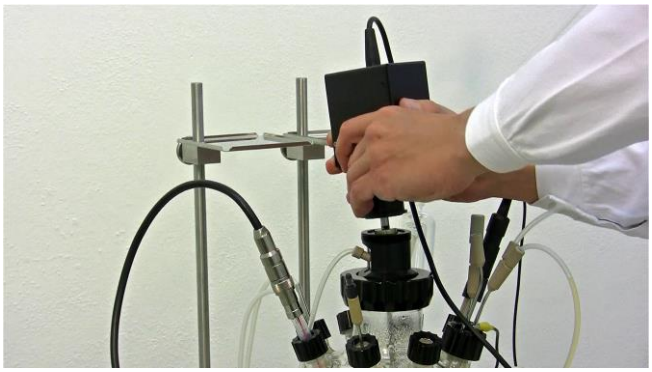


Figure 3.15—2 With the air-input mobile PEEK cock in the upper position; place the vibromixer on the agitation head unit as shown. The magnets will be engaged tightly holding the vibromixer and agitation unit in position.

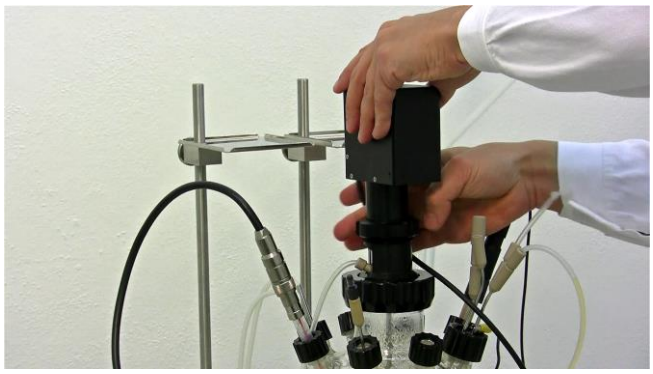


Figure 3.15—3 Once the magnets are engaged, the air-input with the mobile PEEK cock displace downwards. Secure the vibromixer firmly by screwing the cap.

Figure 3.15—4 Connect the agitation unit cable to the "MIXER" socket present at the left side of the MINIFOR Base Unit.

3.15.3 pH and temperature probe

The pH and temperature probe can be connected with the help of the pH probe connector (black cable) present at the front upper left position of the MINIFOR base control unit.

The female plugs of cables cannot be cleaned and must be kept absolutely clean.

Do not touch the tips of the pH-temperature probe and the probe connector.

Figure 3.15—5 Take the pH probe connector from the base unit of the MINIFOR Bioreactor. (Black cable from the base unit).
Remove the black protection cap on the sensor (top part) of the cable.

Figure 3.15—6 Remove the protective cap from the pH probe (red cap)

Figure 3.15—7 Connect both the pH probe connector cable from the MINIFOR base unit with the pH probe. Rotate and adjust the connector cable for exact locking of grooves.

Figure 3.15—8 After locking the pH probe and connector cable; tighten the connection by screwing the threaded lock on the connector cable by rotating as shown.

3.15.4 Peltier Cooling finger for medium

The device is **best suited for 1L vessels, maximal 3L vessels.**

Do not install the Peltier medium cooling device on 7L MINIFOR vessels.

The optional Peltier cooling finger allows the cooling of the working medium without using the cooling liquid and it has to be connected to any pump socket at the rear of the MINIFOR fermenter-bioreactor or connected to any socket of the quadruple plug box (art. no. 800202).

Figure 3.15—9 Peltier cooling finger can be directly connected on the “PUMP”socket present at the rear of the MINIFOR base control unit.

Figure 3.15—10 The quadruple plug box can be connected to the “PUMP”socket at the rear of the MINIFOR base unit. The Peltier cooling finger can be connected to any of the four sockets in quadruple plug box.

For multiple use of the PUMP connector, you can purchase the **quadruple plug box** (see LAMBDA Laboratory price-list).

3.15.5 Peristaltic Pump and MASSFLOW

The LAMBDA Peristaltic pumps and MASSFLOWs can be connected to the MINIFOR base control unit. The connection of Pumps / MASSFLOW with the MINIFOR’s central power supply has to be done by the pump connection cable (art. No. 4810) with round male eight pin connectors on both the ends of the cable.This connection provides the automatic RS-485 interface connection to the system.

LAMBDA Peristaltic Pumps / MASSFLOW can be used independently with the help of the power supply (art. no. 4820 or 4821).

Make sure that you **remove the 12V DC connector before you connect the Pumps / MASSFLOW with the MINIFOR!**

Figure 3.15—11 Pump remote control (analog and digital) cable, 8 poles (open ends) [Art. No. 4810] should be used to connect the Pumps / MASSFLOW to the MINIFOR base control unit.

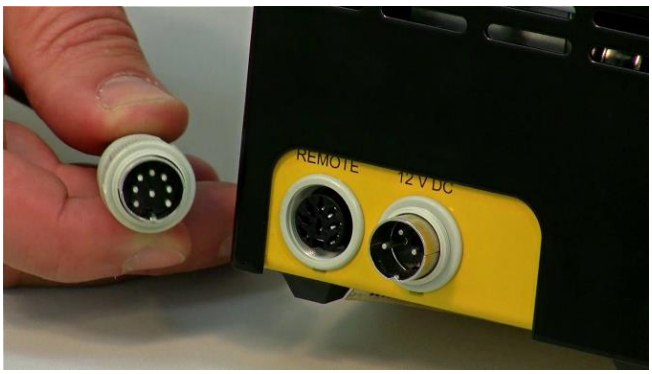


Figure 3.15—12 Connect one end of the remote control cable to the corresponding “REMOTE” sockets at the rear of the peristaltic pump or MASSFLOW.

Figure 3.15—13 For automatic pH control, connect the pump / MASSFLOW that controls alkalinity of the reaction to the “BASE” socket at the rear of the MINIFOR base control unit.

Figure 3.15—14 Connect the Peristaltic pump that regulates the acidity of the reaction to the “ACID” socket at the rear of the MINIFOR base control unit.

3.15.6 pO_2 probe

The pO_2 probe has to be connected with the help of the two way pO_2 (DO) probe connector. pO_2 probe connector need to be connected between the pO_2 probe and the pO_2 (DO) probe socket present in the front upper right of the MINIFOR base control unit.

Figure 3.15—15 pO_2 (DO) probe connector will be connected between the pO_2 probe and the pO_2 (DO) probe socket.

Figure 3.15—16 Adjust the sleeve of the pO_2 (DO) probe connector by pushing it up as shown.

Figure 3.15—17 Place and adjust the pO_2 (DO) probe connector by rotating to find the position for exact locking of grooves with the pO_2 (DO) probe.

Figure 3.15—18 After fixing the pO_2 (DO) probe and connector exactly in locking position, push down the sleeve of the pO_2 (DO) probe connector and secure it sufficiently with screw fitting.

Figure 3.15—19 Remove the protection cap by pressing and unlock by rotating from the pO_2 (DO) probe socket on the base control unit.

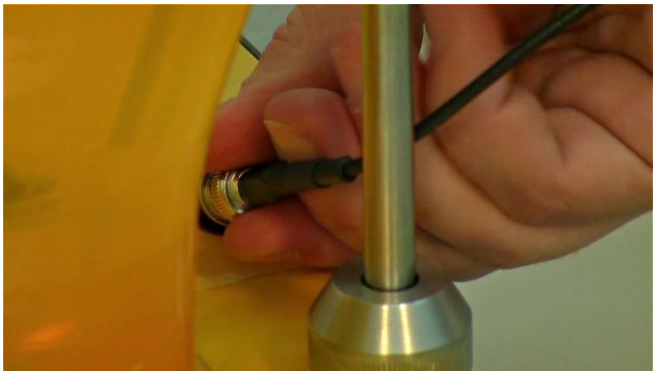


Figure 3.15—20 Secure the pO_2 (DO) probe connector into the socket by rotating and pressing to lock it together.

Do not clean the female plug of the probe cable! (It must be kept absolutely clean)

Do not touch the tip of the pO_2 probe and the connection points of the cable.

3.15.7 Gas flow inlet

The gas (air, O_2 or gas-mix) for the aeration has to be connected by gas tubing to the corresponding socket at the rear of the MINIFOR base control unit.

Make sure to use **pressurized gas < 0.2 MPa.**

Make sure that the **gas pressure is constant**.
Pressure fluctuation can be reduced by using a gas valve between gas cylinder and MINIFOR or between the tubing and MINIFOR

It is possible to use LAMBDA **AEROSILENTO compressor** for gas supply.

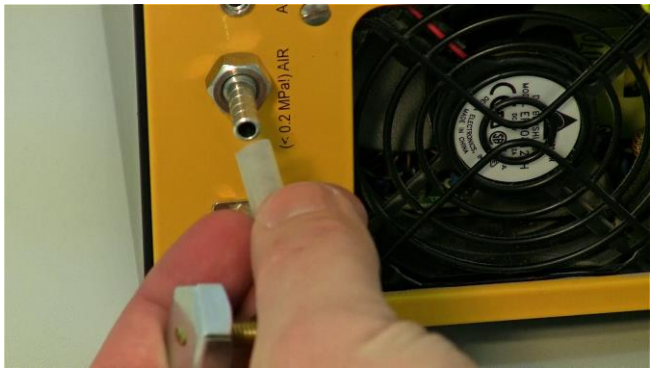


Figure 3.15—21 Connect the air tubing connector that provides pressurized gas < 0.2 MPa, on the rear of the MINIFOR base control unit. The connection socket will be labelled as (< 0.2 MPa) AIR to avoid confusion.

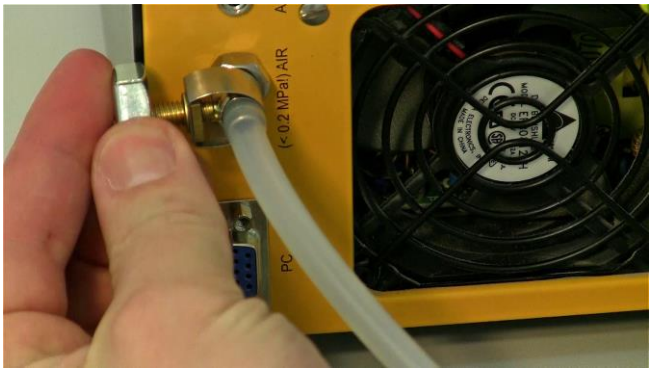


Figure 3.15—22 Secure the connection using the valve.

3.15.8 Gas flow outlet

The gas outlet need to be condensed using the glass condenser available with both the advanced and the start-up kit. The glass condenser used for condensing the outgas using cooling liquid

The optional Electronic Peltier outgas condenser used without the cooling liquid based on the thermoelectric effect of Peltier cell has to be connected to any pump socket at the rear of the MINIFOR fermenter-bioreactor or connected to any socket of the quadruple plug box (art. no. 800202).

For multiple use of the PUMP connector, you can purchase the **quadruple plug box** (see LAMBDA Laboratory price-list).

Figure 3.15—23 Peltier outgas condenser can be directly connected on the “PUMP”socket present at the rear of the MINIFOR base control unit.

Figure 3.15—24 The quadruple plug box can be connected to the “PUMP”socket at the rear of the MINIFOR base unit. The Peltier outgas condenser can be connected to any of the four sockets in quadruple plug box.

3.15.9 Parameter X

The optional weighing module for the continuous fermentation has to be plugged into the socket “X”on the left side of the MINIFOR base control unit (next to the agitation unit connection socket “MIXER”).

Figure 3.15—25 Connect the weighing module connection cable in the "X" socket beside the “MIXER” on the left side of the MINIFOR base control unit.

Figure 3.15—26 Connect the corresponding pump (for the addition or removal of medium) to the “PUMP X” socket on the rear of fermenter-bioreactor MINIFOR.

3.15.10 ANTIFO and DOZITO

DOZITO syringe pump has to be connected with the ANTIFO anti-foam controller. ANTIFO’s 8-pole plug needs to be plugged into the “PUMP” socket at the rear of the MINIFOR base control unit.

For multiple use of the PUMP connector, you can purchase the **quadruple plug box** (see LAMBDA Laboratory price-list).

Figure 3.15—27 The two crocodile clips of the ANTIFO need to be connected with the cannulas from the quadruple sampling port for the detection of the foam level in the working vessel.

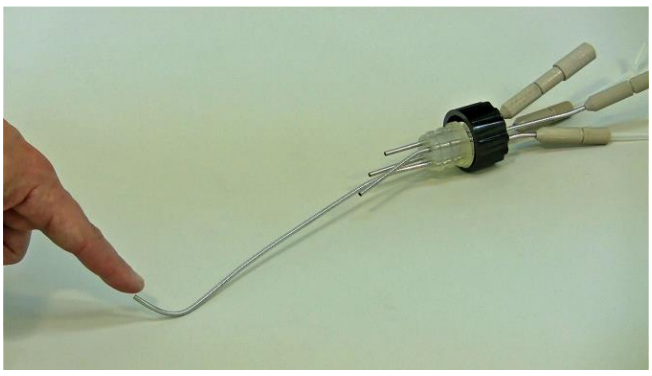


Figure 3.15—28 The harvest needle of the quadruple sampling port (which reaches to the bottom of the vessel) is used as the medium contact.

Figure 3.15—29 Connect one of the crocodile clips to the harvest needle of the quadruple sampling port.

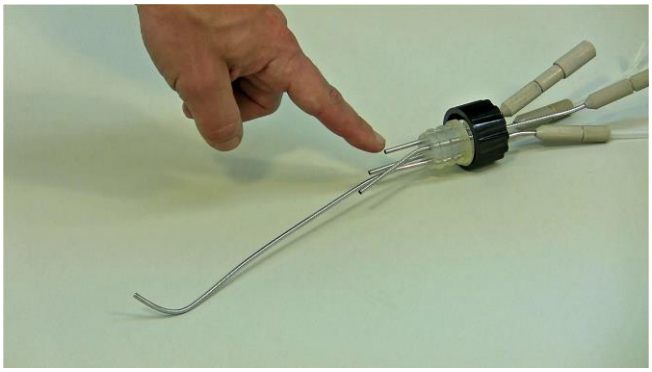


Figure 3.15—30 The second (foam) crocodile clip can be connected to any one of the three shorter needles of the quadruple sampling port.

Figure 3.15—31 Connect the second crocodile clip to the selected short needle.

Figure 3.15—32 Plug in the DOZITO's black cable to the black cable of ANTIFO, as shown.

Figure 3.15—33 Connect the grey colour ANTIFO cable (8 poles) to the "PUMP" socket present on the rear of the MINIFOR base control unit.

3.15.11 Grounding the MINIFOR

Grounding (or earthing) of MINIFOR has to be done to prevent the accumulation of static electrical charges and also for the safe handling of the instrument.

Figure 3.15—34 The black cable with the yellow crocodile clamp is used for grounding.

Figure 3.15—35 Connect the black cable with green connector to "GND" at the rear of the MINIFOR base unit for grounding (or earthing).

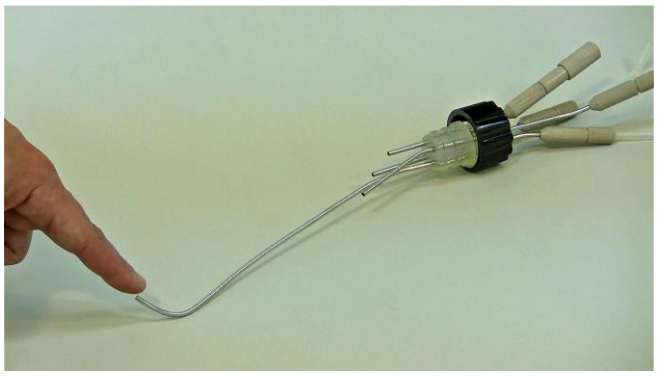


Figure 3.15—36 The grounding (or earthing) cable has to be connected with the longest cannula in the quadruple sampling port.

Figure 3.15—37 Clamp the other side of the grounding cable with yellow crocodile clamp to the longest cannula of the quadruple sampling port.

If possible, connect the **grounding cable to the needle of the sampling port** which is dipped deep into the medium. This will stabilize the readings of both the pH and pO_2 probes. Both have high impedance amplifiers and probes can thus function as antennas and pick up all kinds of ‘electrosmog’ in your lab.

3.15.12 PC connection

The PC is connected to the rear of the MINIFOR base control unit with a DB 9 connector. All the necessary parameters can be controlled from PC using a fermentation control software FNet or SIAM. It enables data acquisition and real-time visualization of the different parameters.

Since most of the PC have serial output with the RS-232 interface, a converter RS-232/485 is necessary. The RS-485 allows connection of dozens of different instruments by the same line, whereas with RS-232 only one instrument can be controlled. A corresponding converter is part of the fermentation software FNet or SIAM.

LAMBDA MINIFOR can be connected to the PC serial port through the RS-232/485 converter.

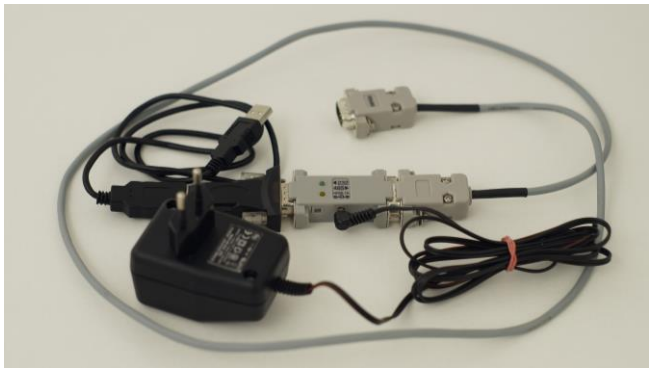


Figure 3.15—38 PC connection kit with RS-485 to RS-232 converter and USB connection.

Figure 3.15—39 Connect the PC connection cable to the DB 9 connector at the rear of the base control unit.

3.15.13 Alarm

Each parameter has its individual alarm based on the set low and high alarm values.

When the alarm is activated, a continuous 12V signal is present on the alarm output. This is very helpful for directing the alarm to other places, e.g. by phone or activating a sample collection with the aid of the sample collector OMNICOLL. Such samples can contribute to clarification of alarms during unattended fermentation.

Figure 3.15—40 Alarm connector cable can be used to direct the alarm to other places, e.g. phone

Figure 3.15—41 The alarm connector cable plug has to be connected into the alarm socket at the rear of the base control unit. Other end of the cable can be connected to the desired devices.

3.15.14 Main power supply

MINIFOR's main power supply cord has to be connected to the power inlet at the rear of the MINIFOR base control unit (100-230V/50-60Hz) and the system can be switched on and off using a small black switch present just below the power supply connection.

Do not plug in the power supply, unless you are done with all other connections.

Checklist before switching on the MINIFOR fermentor-bioreactor:

- Air inlet
- Pumps, MASSFLOW, Weighing Module, ANTIFO
- pH and pO_2 probe

- PC connection
- Grounding (Earthing)
- In case of surface aeration:
    - ➔ Butterfly shaped stirring discs for minimal working volume, should not use sparging.
    - ➔ Disconnect the air inlet (Pressurized air <0.2 MPa)
    - ➔ Check the installation of surface aeration, clamp the tubing leading to sparger.
    - ➔ Make the air flow control = 0, once switched on

Make sure that Pumps / MASSFLOW gas controllers are not powered by an external power supply separately!
The used 12V voltage of the external power supply and the MINIFOR power supply will not exactly be the same and current may flow from one instrument to the other. This could generate problems and damage the instruments. If such a connection is inevitable a diode in the power supply line would be needed.

Figure 3.15—42 Main power supply cord. (Shown is the European power plug. Power plug will be supplied according to the country).

Figure 3.15—43 Connect the Power cord to the power inlet (100-240 V/ 50-60 Hz) at the rear of the MINIFOR base control unit.

Figure 3.15—44 Once the connections and installations are done, switch on the MINIFOR using the power switch present below the power input. (Power Switch: I = On; O = Off)

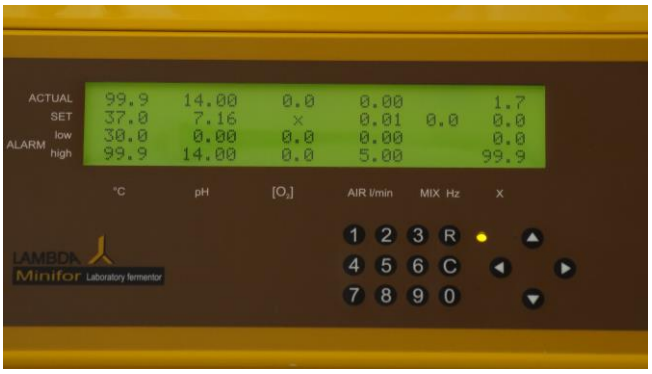


Figure 3.15—45 When switched on, the MINIFOR starts with a beep sound and yellow LED display on the control panel. This represents the Stand-by mode of the MINIFOR fermentor-bioreactor.

3.16 Peristaltic Pump Installation

Once the MINIFOR is switched on, the pumps connected to the MINIFOR base control unit will be switched on by the central power supply.

Make sure that Pumps are not powered by an external power supply separately!
The used 12V voltage of the external power supply and the MINIFOR power supply will not exactly be the same and current may flow from one instrument to the other.

This could generate problems and damage the instruments. If such a connection is inevitable a diode in the power supply line would be needed.

3.16.1 Installation of tubing

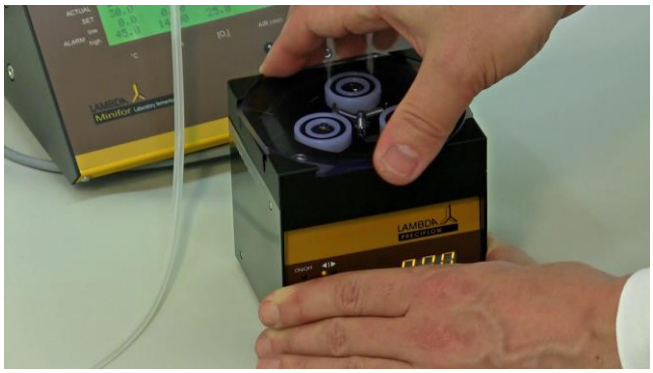


Figure 3.16—1 For the installation of tubing into the Peristaltic pump, twist the transparent PVC head cover either clockwise or anti-clockwise to remove.

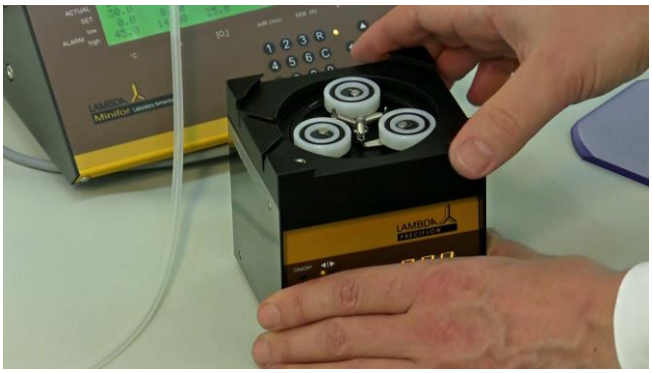


Figure 3.16—2 Remove the transparent PVC head cover. The speed of rotation has to be increased using the **"Λ Λ Λ"** arrows present below the LED display.

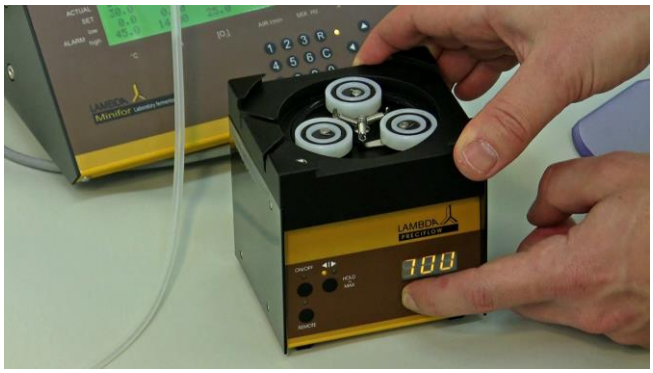


Figure 3.16—3 Set the pump speed to about 700. The speed range of about 300-700 for tubing insertion can be used.

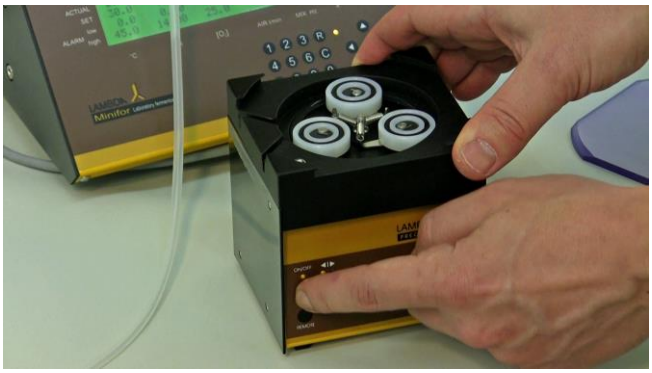


Figure 3.16—4 Press the ON/OFF button and select the sense of rotation of the pump by pressing the button ◄I►

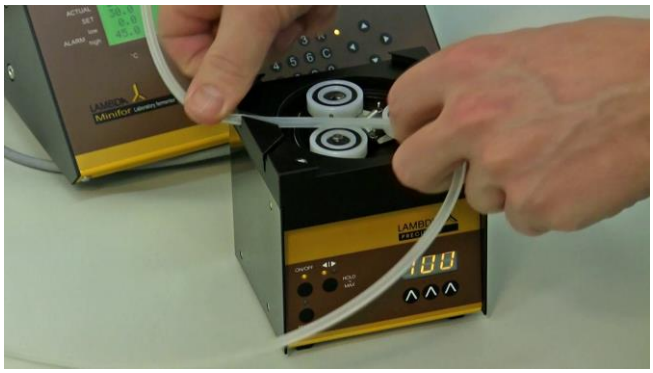


Figure 3.16—5 Press the tubing into the back slot on the top of the peristaltic pump. Thin tubing should be pushed completely to the bottom of the slot.

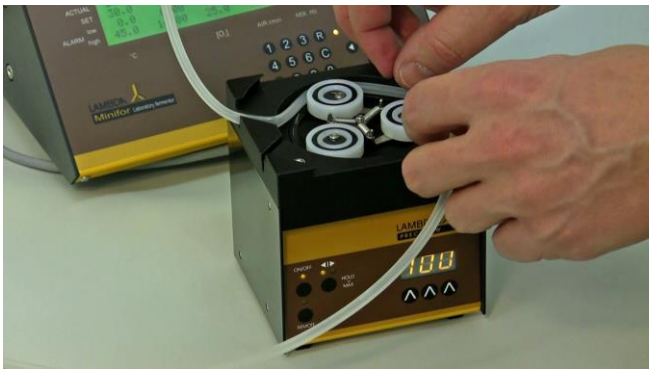


Figure 3.16—6 Guide the tubing around the slowly turning plastic bearings towards the front slot.

Figure 3.16—7 Press the tubing into the front slot to secure it.

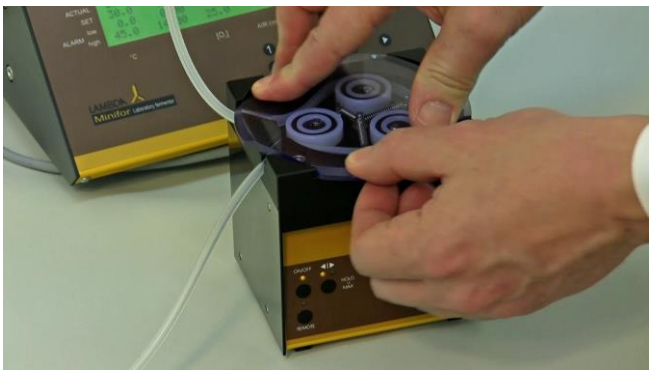


Figure 3.16—8 Replace the transparent PVC head cover and twist it gently to secure it.

Figure 3.16—9 Make sure that the steel ball embedded in the left-front corner fits into the corresponding notch in the PVC head cover.

If the LED above the **REMOTE** button is on, then the pump is **remotely controlled by MINIFOR or FNet.**

Make sure that you **do not switch on the REMOTE** control **without checking the pump line** and the **MINIFOR / FNet settings**. It prevents mistakes and accidents.

For the remote control of a pump (such as acid or base pump) by MINIFOR, the pump has to be plugged in correctly and then:
- **REMOTE** and **ON/OFF LED** should be **yellow**.
- If the pump **ON/OFF LED = yellow** and **REMOTE LED is not illuminated** then the pump will have to be **controlled locally** over the display.
- If the pump is switched off (**ON/OFF LED = dark**) and **REMOTE CONTROL** is on (**LED yellow**) -> beware nothing gets pumped.

3.16.2 Setting up of the flow rate

The flow rates delivered by peristaltic pumps depend on the internal diameter of the tubing and the pump rotation speed. The speed of rotation can be selected by the control buttons Λ Λ Λ below the LED display.

With the control buttons Λ Λ Λ below the LED display the motor speed is selected. The speed setting from 0 to 999 corresponds to the velocity of the movement of the motor. The best way to correlate the flow rate obtained with the respective tubing is to make a preliminary calibration, in which the pump is allowed to pump the liquid over a certain time with a selected speed setting (e.g. for 1 minute with speed setting 500). Then, the weight or volume of the pumped sample is measured. Using this information the speed setting corresponding to the desired flow rate can be calculated easily (rule of three).

Figure 3.16—10 The **Λ Λ Λ** buttons can be used to set the desired flow rate.

Figure 3.16—11 Each **Λ Λ Λ** arrow can be used to change the corresponding digit on the flow rate.

If you need to know the amount of liquid added without a balance or if you want to measure the flow rate, then you will need the calibration of your LAMBDA pump speed / flow rate.
However, the inner diameter of the silicon tubing can change due to sterilization heat. The first sterilization has the biggest impact on the material. **Use only AUTOCLAVED tubing for calibration and use the same tubing that you are going to use during fermentation.**

The speed of the pump can be controlled on the pump LED display, while the pump is connected to MINIFOR or FNet.
Therefore, you have to make sure that the remote control is off: the REMOTE-LED is not illuminated. (You can switch off by using the REMOTE button on the LAMBDA peristaltic pump)

3.16.3 Choosing flow direction

The direction of the pump rotation can be selected by the ◄I► button, clockwise or anti-clockwise. The corresponding direction LED diode will be on.

If possible use the **clockwise rotation** of the tubing pump. This results in **lower friction** and the **pressure of the liquid** (approx. 0.1 MPa). If a **higher pressure is required** (up to 0.15 MPa), use the **counter-clockwise rotation**.

Figure 3.16—12 LED illumination in the direction ◄| represents the clockwise rotation.

Figure 3.16—13 LED illumination in the direction I► represents the anticlockwise rotation.

3.16.4 Fast filling or emptying the line

If the direction ◄I► button is pressed continuously for about 2 seconds, the pump will rotate at a maximum speed in the direction of rotation indicated by the LED. “HOLD = Maximum”

After releasing this direction button the pump will stop pumping. This factor can be used for filling tubing before starting or for emptying the tubing line at the end of the operation.

The calibration of the pump flow rate with speed can be done to know the amount of the liquid added in the reaction vessel. For calibration, refer 5.4 Pump Calibration.

3.16.5 Connection of Peristaltic pumps

The acid and base pump (MINIFOR Advanced kit) for the pH maintenance should be connected to the ‘Acid’ and ‘Base’ socket at the rear of the MINIFOR base control unit. The feed or harvest pump of the weighing module has to be connected to ‘Pump X’ socket. Other pumps can be connected to the Pump socket.

Make sure that Pumps are not powered by an external power supply separately!
The used 12V voltage of the external power supply and the MINIFOR power supply will not exactly be the same and current may flow from one instrument to the other. This could generate problems and damage the instruments. If such a connection is inevitable a diode in the power supply line would be needed.

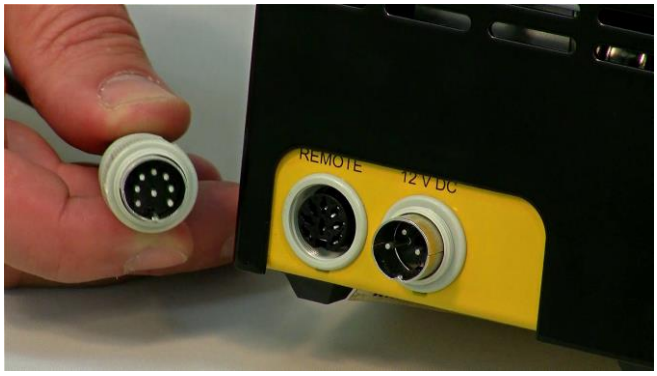


Figure 3.16—14 Connect the 8-poles Pump remote control cable into the 'Remote' socket at the rear of the Peristaltic Pump.

Figure 3.16—15 Pumps has to be connected into the respective sockets at the rear of the MINIFOR base control unit.

Figure 3.16—16 Connect the base Peristaltic pump cable into the 'BASE' socket on the MINIFOR base control unit.

Figure 3.16—17 Connect the acid Peristaltic pump remote cable into the 'ACID' socket on the MINIFOR base control unit.

Figure 3.16—18 Feed or the Harvest pump of the weighing module should be connected to the PUMP 'X' socket.

Figure 3.16—19 Other additional pumps can be connected to the PUMP socket.

For multiple use of the PUMP connector, you can purchase the **quadruple plug box** (see LAMBDA Laboratory price-list).

3.16.6 Placement of the Peristaltic Pumps

The Peristaltic Pumps can be placed on the pump supports which is installed on the support rods on the MINIFOR base control unit. In this way, it will be accessible and do not occupy valuable bench-space.

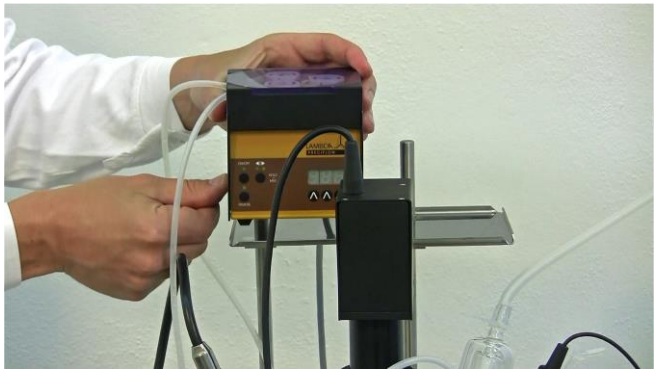


Figure 3.16—20 The Peristaltic pump can be placed on the pump support which is installed on the support rods on the MINIFOR base control unit.

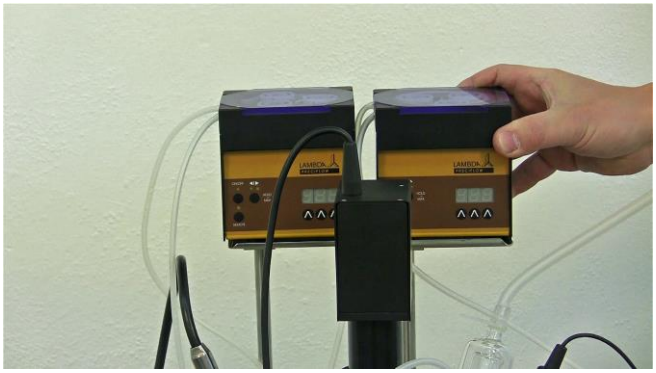
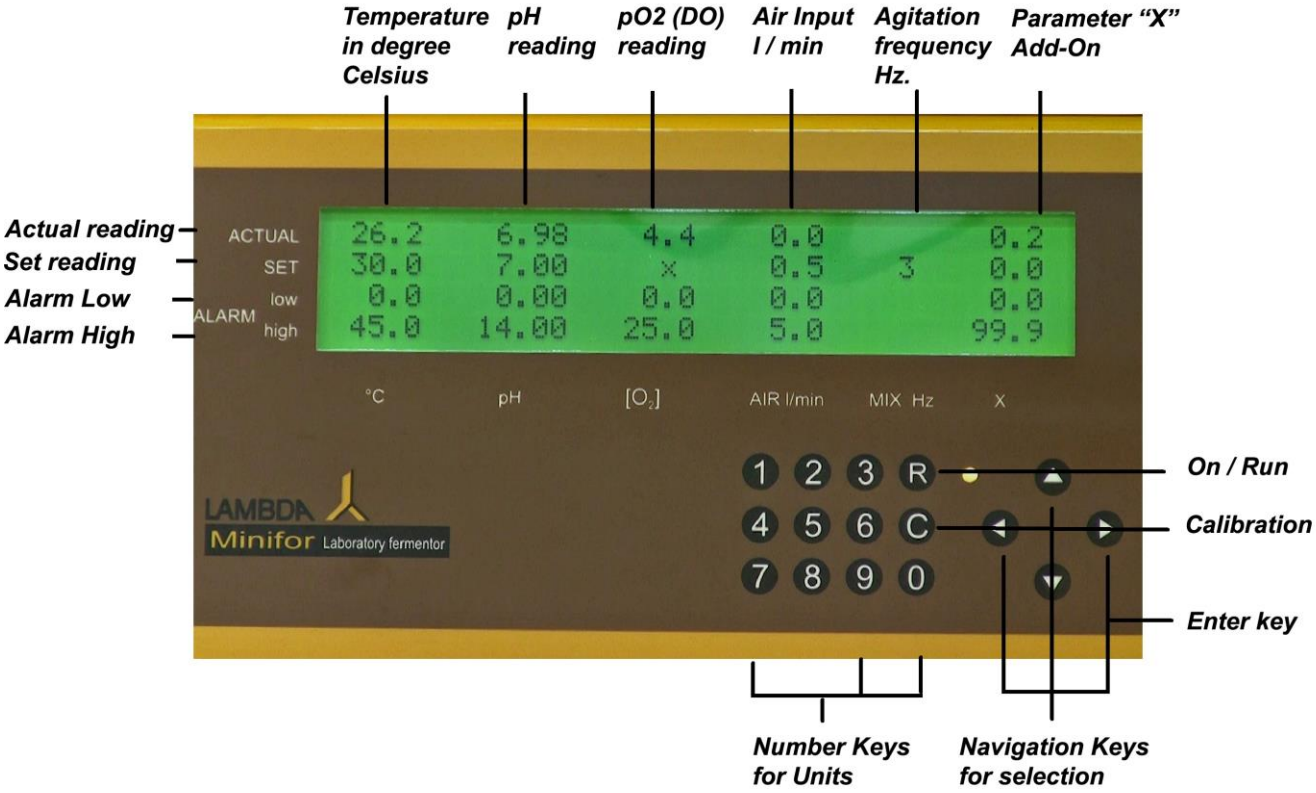


Figure 3.16—21 Placing the other Peristaltic pump on the pump support.

4 Display and control panel

The control panel of the MINIFOR base control unit consists of a display with keyboard.



Keys and Display (standby mode): all values are seen on one screen without any scrolling.

There are three categories of keys:

- Function Keys
- Navigation Keys
- Number Keys

The display shows 3 different modes (states of MINIFOR):

- Standby
- Regulation (Operation)
- Calibration

When mode of operation is changed on the MINIFOR then the display will be used for that mode. All values of the mode are shown on the display. It is necessary to scroll up and down.

Input modification:

Place the cursor on the desired parameter using the "ENTER KEY". Enter the value with the number keys. The values need to be entered without any decimal point. For example, 9.00 have be entered as 900. After entering the value using the number keys, press the enter key. The enter key will show the actual value with the decimal point on the display and the value will be saved.

Input modification is always the same, independently of the operating mode of the fermenter.

4.1 Keys

The MINIFOR display is equipped with a keyboard that contains number keys, navigation keys, C and R.

Table 10 MINIFOR Keys on the front panel and their functions

Keys on MINIFOR front panel			
Function Keys	Safe calibrations Switch between states (Standby, Calibration, Regulation)	**C**	○ Switch from Standby to Calibration/ configuration mode ○ Save the calibration of each parameter to be used
		R	○ Change the Calibration to Standby mode ○ Switch between Standby and Regulation configuration (RUN with REGULATION)
Navigation Keys	Cursor	**Arrow keys: <, >, ∧, v**	○ Activates and moves the Cursor
	Clear value	**<**	○ Clears the value (set-point, calibration value) allowing correction
	Save value (Enter)	**>, ∧, v**	○ Save the entered value (set-point, calibration value)
Number Keys	Write values	**0, 1, 2, 3, 4, 5, 6, 7, 8, 9**	○ Entering values

4.1.1 Function keys (C, R)

Table 11 MINIFOR Function Keys

C	○ Switch from Standby to Calibration / **configuration** ○ Save the calibration of each parameter to be used
R	○ Change the Calibration to Standby configuration ○ Switch between Standby and Regulation state (RUN with REGULATION)

START/STOP- or STANDBY-Key **"**R**",** activates and deactivates the regulation and alarms. The measured values are not influenced thereby and the actual values are displayed. This option is helpful during the calibration of the probes and the preparation of the fermenter. In standby mode the LED is yellow. In operation mode it is green.

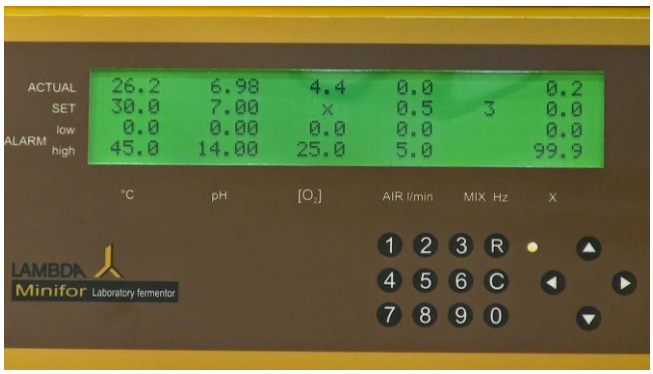


Figure 4.1—1 Stand-by mode of the MINIFOR. “R” key used to switch between Standby and Regulation state (RUN with REGULATION)

Figure 4.1—2 "C" - Calibration key is pressed to change the stand-by mode to calibration mode.

Figure 4.1—3 Calibration mode.

4.1.2 Navigation Keys (Arrow Keys)

- Pressing will **activate the cursor** on the display. The cursor is represented by a blinking field under the activated digit.
- If the cursor is already activated, pressing the arrows will **move the cursor** to the corresponding direction.
- If a value is being edited (e.g. calibration value) the left arrow **clears the value** allowing correction, whereas the **other arrows save the entered value** and move the cursor to the corresponding direction.
- If the cursor leaves the field (arrows right, up or down) the modified value will be saved automatically.

Figure 4.1—4 Press the "C" - calibration key continuously to activate the cursor.

Figure 4.1—5 The cursor is represented by the blinking of the activated digit.

Figure 4.1—6 Once the cursor is activated after the long press of “C” key, pressing the arrows will move the cursor to the corresponding direction.

Table 12 MINIFOR Navigation keys

Cursor	**Arrow keys: <, >, ∧, v**	Activates and moves the cursor
Clear value	**<**	Clears the value (set-point, calibration value) allowing correction
Save value (Enter)	**>, ∧, v**	Save the entered value (set-point, calibration value)

The cursor will be deactivated automatically, if no change done using number keys or arrows in the time interval of 15 seconds.

4.1.3 Number Keys

The number keys 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 0 are used for changing the values (values = set-points, alert limits, calibration values etc.)

If no cursor is displayed, the number keys are blocked from entering the values, preventing the inputs by mistake.

Figure 4.1—7 The number key is used to change the value of the blinking cursor.

Figure 4.1—8 Changing the value of the selected parameter using number keys.

4.2 Modes (states) of MINIFOR

MINIFOR has three states (modes):

Figure 4.2—1 Standby mode - state of the fermenter after switching on. The display shows the last entered parameters. In this configuration all measurement readings are shown, but the regulation is switched off and the number keys blocked. LED is yellow and all numbers on the display are static.

Figure 4.2—2 Regulation / Operation mode - The regulation is activated by pressing key “R” and the LED turns to green. The display shows the measurement of all the parameters.

Figure 4.2—3 Calibration mode - Key “C” starts the calibration of pH, pO_2, parameter X or the setting of the address for PC control (depending on the choice with the cursor). The LED is yellow and the regulation is off. (Only the agitation is activated to allow precise calibration of pO_2 probe).

The actual state of MINIFOR can be recognized by the display of the following on the MINIFOR Control Panel and LED.

Table 13 States (Modes) of MINIFOR display

State (mode)	Activation Key	LED	Parameter calibration	Parameter Measurement	Parameter control	Number Key
Standby	R	yellow	-	All parameters: °C, pH, [O2], AIR l/min, MIX Hz, X	off	Blocked until arrow keys activate the **cursor**
Operation	R	green	-		on	
Calibration	C	yellow	pH, pO_2, X, PC connection		Stirring on, all others off	Activated

The MINIFOR modes have no influence on the cooling (cooling loop as well as the peltier cooling finger)!

With the help of the function keys on the MINIFOR display, the configurations (modes) can be changed.

4.3 SET-point and ALARM

For each parameter on the MINIFOR display, the SET-point as well as the ALARM limits can be chosen. The SET-points as well the ALARM limits (low and high) can be changed in the Regulation mode and the Standby mode.

- **SET Point** — The **actual value** that the automatic parameter control should reach.
- **ALARM LOW** — The **minimal ACTUAL value** that the automatic parameter control is allowed to reach.
  If the ACTUAL value of the parameter is lower than the value entered as ALARM LOW then an * will occur beside that ALARM value and depending on your setting, an acoustic alert will be activated.
- **ALARM HIGH** — The **maximal ACTUAL value** that the automatic parameter control is allowed to reach.
  If the ACTUAL value of the parameter is higher than the value entered as ALARM HIGH then an * will occur beside that ALARM value and depending on your setting, an acoustic alert will be activated.

ALARM LOW and ALARM HIGH are not activated (visual * or acoustic) if the corresponding value of ALARM LOW has been set to **0.0 or 0.00** previously. This prevents alarms of unused functions, e.g. parameter X.
For all non-zero values alarm is activated! (e.g. 0.01)

When the alarm is activated, **a continuous 12V signal is available on the alarm output.** This signal is helpful for directing the alarm to other places, e.g. by phone or for activating a sample collection using the sample collector OMNICOLL. Such samples can contribute to the clarification of the alarms during an unattended fermentation.

If the **ALARM is activated** (it means a value higher than 0.00 has been entered in the ALAMR LOW), then the ALARM alert will be shown on the **Standby and Regulation mode** – not in Calibration mode!

4.3.1 Setting the temperature

Figure 4.3—1 Move the cursor by using arrows on the field for the temperature setting (SET °C, second row, first column).

Figure 4.3—2 Enter the value for the desired temperature (the value shown on the first line and first column corresponds to the measured temperature and cannot be changed).

Figure 4.3—3 Move the cursor on the field of temperature minimum (ALARM LOW). Enter the desired value. If the temperature falls below this value, an alarm is activated and an asterisk is displayed on the left of the corresponding value.

Figure 4.3—4 Move the cursor on the field of temperature maximum (ALARM HIGH). Enter the desired value. If the temperature exceeds this value, an alarm is activated and the exceeded value is highlighted on the display.

The cursor will be deactivated automatically, if no change done using number keys or arrows for the time interval of 15 seconds.

4.3.2 Setting the pH, pO_2, Air-flow, stirrer, X

The parameters pH, pO_2, air-flow, stirrer and X set analogically same as that of the temperature (see the chapter above 4.3.1 Setting the temperature).

Choose either the automatic pO_2 control or the automatic air-flow. Since the internal MASSFLOW controls both the parameters, they cannot be controlled simultaneously.
If you wish to control both parameters simultaneously, then an additional external MASSFLOW can be used, as well as the additional controlling tools by the optional PC fermentation control software.
For further information, you may contact LAMBDA Laboratory Instruments at support@lambda-instruments.com.

Figure 4.3—5 Move the cursor using the navigation key and place it on the MIX Hz column. Set the desired frequency of agitation using the number keys and press the right arrow for saving the entered value.

Do not try to include the ALARM limits for the parameter MIX (stirrer frequency), since that option does not exist.
For the stirring function (MIX) only the desired value can be entered. The actual value corresponds automatically to the SET-point value, because the frequency of mixing is controlled precisely through the electronics and deviation thereof is not possible.

The concentration of dissolved oxygen (pO_2) is regulated by continuous variation of airflow. This regulation is activated by entering a value for pO_2 into the field of the desired pO_2 value. The symbol “x” appears on the display at the location of the desired airflow to point out that the airflow is determined by pO_2 controller alone. The measured airflow value is shown and corresponding alarms (ALARM HIGH, ALARM LOW) can be set. To return to the airflow regulating mode, move the cursor on the column of the airflow and enter the desired value. The symbol “x” is now displayed at the location of the desired pO_2.

4.3.3 Alert (de)activation

The alarm can be deactivated by entering the corresponding ALARM LOW value in the appropriate parameter column.

For example: Activating and deactivating the high and low alarms for the temperature.

Figure 4.3—6 Alarm high is activated. * sign can be seen with the alarm high value.

Figure 4.3—7 Alarm can be deactivated by changing the alarm high value and by taking needed precautions to maintain the temperature.

Each parameter has its own ALARM. If you need to be indicated with more than one alarm, you have to enter 0.00 in ALARM low for the corresponding parameter. The alarm of a parameter is not activated, if the corresponding value of ALARM LOW has been set to 0.0 or 0.00 previously. This prevents alarms of unused functions, e.g. parameter X.

For all non-zero values the alarm is activated! (e.g. 0.01)

For Temperature ALARM low and high, do not put any ALARM closer than 0.3 °C to the set-point, since the accuracy is ±0.2°C (0 to 60°C)

5 Calibrations

5.1 Stirrer, Temperature, Flow rate: No calibration

Temperature probe, flow meter and the stirrer frequency cannot be calibrated. Their actual values are guaranteed and controlled electronically.

If you would like to change it, then contact LAMBDA Laboratory Instruments. (www.bioreactors.eu or www.lambda-instruments.com)

5.2 pH probe calibration (gauging)

The calibration of the pH probe has a two-point gauging system with two standard buffers. The calibration mode need to be used – pressing “C” key on the display panel. (Refer 4.2 Modes (States) of MINIFOR)

Refer the leaflet “InPro® 325X, InPro® 325X (ISM), InPro® 325X i” provided along with the pH probe for further information.

The calibration of the pH has to be done BEFOR STERILIZATION!

Choose the buffers according to the pH range of your fermentation.
For standard fermentations with pH ranging between 6.2 and 7.0, we recommend to choose the buffer pH7.0 and buffer pH 4.0 for calibration.

While handling corrosives, put on your goggles and gloves and follow the safety instructions of your laboratory!

5.2.1 List of consumables

- 2 buffers for pH calibration:
  Buffer pH 7.0
  Buffer pH 4.0 or any other buffer
- Glass beaker
- Rinsing water (Deionised water)
- Tissue or blotting paper for drying the probe

5.2.2 Step by step Calibration of the pH

The following step by step calibration of the pH probe is an example of the pH range chosen. The calibration has to be done with the appropriate pH range needed for the reaction. The steps will remain the same but the pH solutions / buffers will differ.

It is necessary to **observe the sequence exactly as described**, to calibrate the probe correctly.

Calibration can be stopped at any moment by pressing the **regulation key "R"**. If the calibration is interrupted, the values of the former calibration remain unchanged. (For cross checking, the probes can be immersed in the gauging solutions and the correct values should be displayed in the standby mode).

Check the connection of pH probe with MINIFOR before calibration!

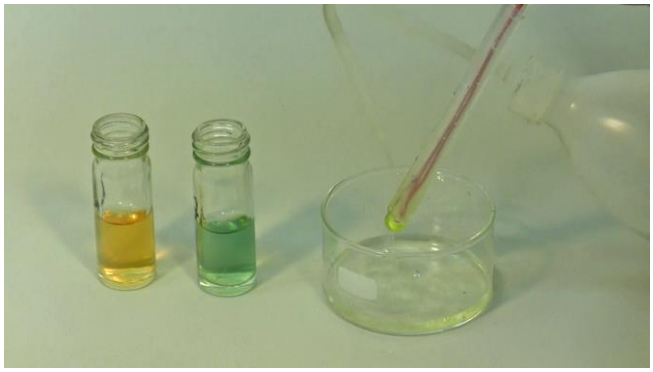


Figure 5.2—1 Prepare the needed buffers and rinse the probe with deionised water. Dry the probe with the blotting paper.

Figure 5.2—2 Change the stand-by mode into calibration mode by pressing the calibration key "C".

Figure 5.2—3 Continuous pressing of "C" key will activate the cursor of the set value of pH.

Figure 5.2—4 To start the gauging of the first pH, enter the desired pH as the set value using the number keys.

For example: Enter 400 for gauging pH 4.

Figure 5.2—5 Press the right arrow "**>**" to SET the blinking value. Please note that after pressing the right arrow, the value 400 changes to 4.00.

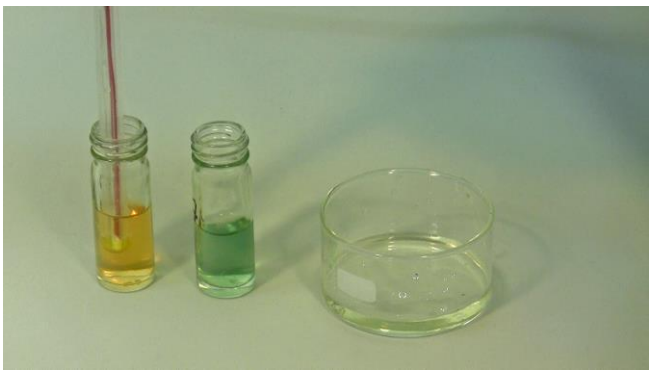


Figure 5.2—6 Dip the probe into the gauging solution (pH 4 buffer solution) and stir gently while the pH is been measured.

Figure 5.2—7 The pH of the buffer will be displayed on the ACTUAL value of the pH (above the SET value). Wait until the ACTUAL value of the pH gets stable.

Figure 5.2—8 Once the ACTUAL value of the pH is stable, press the calibration key "C" in order to save the calibration. The calibrated value will be displayed as BUFFER 1 value of pH.

Figure 5.2—9 Wash the probe with deionised water and dry it with the blotting paper.

Figure 5.2—10 Start the calibration of the second gauging solution, by entering the next pH value and then press the right arrow key "**>**" to SET the needed pH value.
For example: Enter 700 for pH 7.

Figure 5.2—11 After pressing the right arrow key "**>**", the value entered (700) will be SET as pH 7.00.

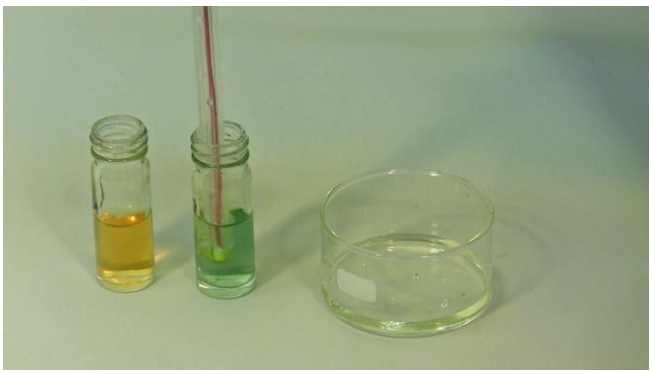


Figure 5.2—12 Dip the pH probe into the pH 7 buffer solution and gently stir it, until the pH value becomes stable.

Figure 5.2—13 Wait until the ACTUAL value if the gauging solution becomes stable.

Figure 5.2—14 Once the value becomes stable, press the calibration key "C". The calibrated value will be saved and displayed as BUFFER 2 pH value.

Figure 5.2—15 OK message will be displayed, if the calibration is done correctly.

Figure 5.2—16 Messages that can appear:

OK - Calibration successful
ERR0 - Same gauging solution used twice
ERR1 - Same value entered twice

If the error message is displayed, the calibration has to be repeated again from the beginning.

5.2.3 Crosscheck the pH calibration

To crosscheck the calibrated probe, use the same pH buffers used for calibration. Press the regulation key "R", once the calibration shows the OK message.

Figure 5.2—17 After pressing the regulation key "R", the MINIFOR changes to the standby mode from the Calibration mode.
Note that the MINIFOR in standby mode or in the operation mode without pumps being activated.

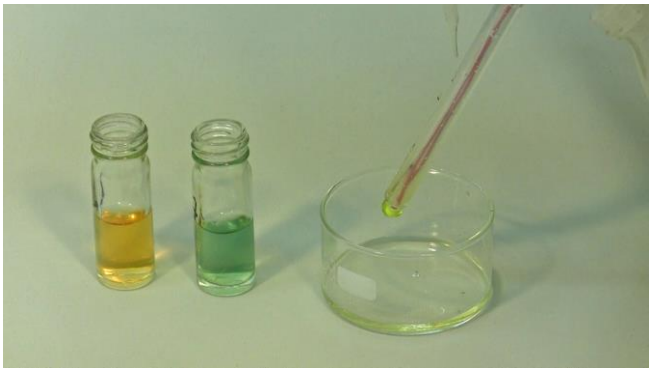


Figure 5.2—18 Rinse the probe with deionised water and dry it with the blotting paper.

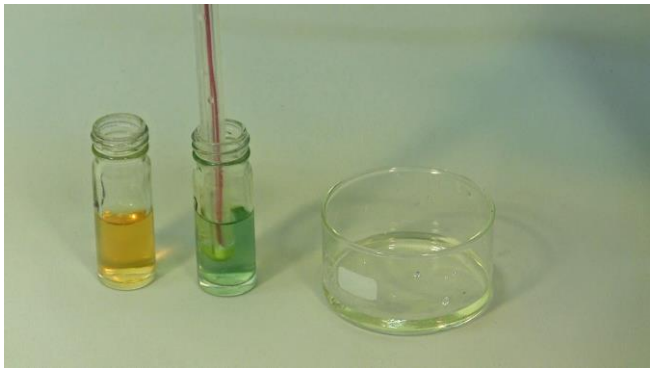


Figure 5.2—19 Test the measurement of the pH into, by dipping the probe into the pH 7 buffer solution.

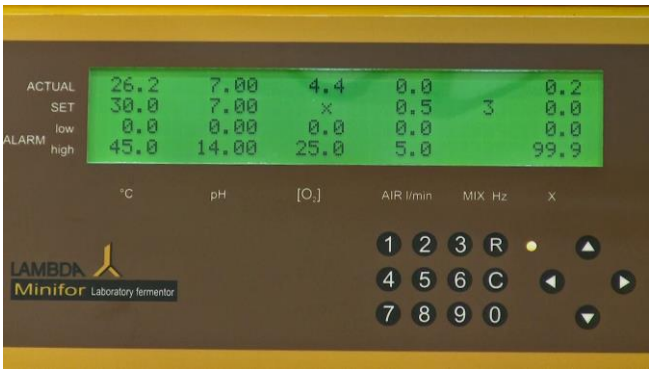


Figure 5.2—20 The ACTUAL value of the parameter pH should be 7.00 (It can range between 6.95 – 7.04)

Figure 5.2—21 Rinse the probe again and dry it with the blotting paper.

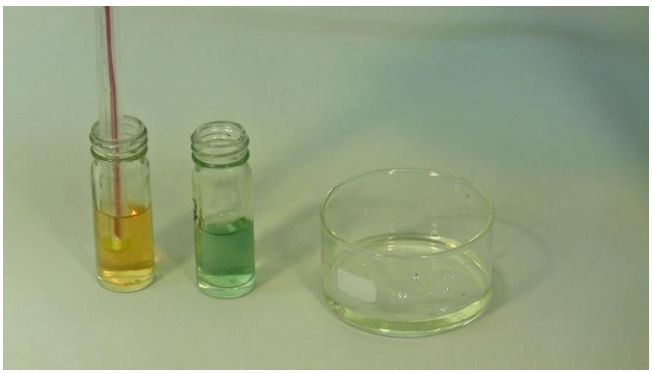


Figure 5.2—22 Now the measurement of pH 4 can be checked by dipping the probe into pH 4 buffer solution.

Figure 5.2—23 The ACTUAL value of the parameter pH on the display should be 4.00 (It can range between 3.96 – 4.04)

If the measurement of the pH 4 and pH 7 during the crosschecking shows a wrong ACTUAL value, then the calibration has to be repeated again.

5.3 pO_2 probe calibration (gauging)

Do you have a pO_2 probe?

The pO_2 probe (DO probe) is included in the MINIFOR ADVANCED KIT. MINIFOR START-UP Kit can get upgraded by an optional pO_2 probe, if needed.

Background information about pO_2 measurement:

The saturating concentration of oxygen in pure water varies with temperature, air pressure and concentration of dissolved substances in the medium.

The variation of the temperature is automatically compensated. (It is however preferable to calibrate the probe at the temperature, that will be used in the run). The calibration is made usually after sterilization with suitable stirring (about 10 Hz). See the table of maximal DO concentrations in function of temperature to find the corresponding value (chapter 5.3.4 Oxygen saturation in water).

Other factors, such as atmospheric pressure and salinity are negligible.

The calibration of the pO_2 probe (DO probe, dissolved Oxygen Probe) is a two-point gauging with two standards.

The calibration and measurement cannot be done without stirring, due to consumption of oxygen by electrochemical process, it is depleted at the proximity of the membrane and the measured signal decreases.

In case you do the calibration in an external vessel, then make sure that the gas bubbles mix well in the solution.

The calibration of the pO_2 (DO) has to be done **BEFORE** and **AFTER STERILIZATION**!

Do the calibration of the probe also **before sterilization**. It will be the best test to show you, if the probe works properly or if the exchange of the membrane and/or electrolyte is needed.

The probe has to be polarized before the measurement and calibration – it also means that the **polarization** of the pO_2 (DO) probe is necessary before each calibration!

5.3.1 List of consumables

- Zero point calibration:
  - 5% aqueous solution of Na_2SO_3 or
  - N_2 saturated water or
  - Zeroing gel for zero-point control (Mettler Toledo) or
- Slope point calibration:
  - Air saturated water
  - Air saturated buffer
  - Air saturated sterile medium (after sterilization)
- Glass beaker
- Rinsing water (Deionised water)
- Tissue or blotting paper for drying the probe
- MINIFOR with pH /Temperature probe
- MINIFOR with pO_2 probe
- pO_2 calibration / Temperature (Table 13)

5.3.2 Inspection of the sensor

The pO_2 electrolyte is an alkaline solution (pH=13).

Avoid contact with skin, especially mucous tissues or eyes. Because contact with the electrolyte very likely occurs during the exchange of electrolyte or membrane module. Usage of protective gloves is highly recommended.
In case of contact, the place should be washed well with plenty of cold water. Get medical attention, if adverse signs appear.

Great care should be taken while handling the glass inner bodies, since any hairline cracks resulting from knocks will adversely affect the sensor performance.

Before each calibration always check the pO_2 probe (DO probe):

- **Check the membrane optically**:
  - Examine the membrane for signs of damage.
  - If damaged then the membrane module of the pO_2 probe needs to be exchanged

- **Is the responding time too long**?
  - If the responding time is too long, then exchange the electrolyte in the pO_2 probe.

- **Sluggish response?**
  - **I**t may be due to the formation of deposits on the membrane. It is possible to clean the membrane with wet soft paper and a small amount of mild detergent. Finally wash the membrane with distilled water.
  - If the cleaning does not help, it may be necessary to replace the membrane.

- **Fast reaction but the signal is unstable?**
    - It may be due to the perforation of the membrane. This also requires replacement.

If the DO probe begins to exhibit signs of failure (long response time, mechanical damage, increased residual current in oxygen free medium, etc.), it has to be replaced!

5.3.3 Polarization

The polarization of the pO_2 probe is absolutely necessary for the proper measurement and calibration of the pO_2.

If the oxygen probe has not been connected to polarizing potential, it will need a certain time to attain its stable signal. This time is called polarization time. It can take an hour or so depending on conditions and time without potential. The LAMBDA oxygen probe has short polarization time.

For the polarization, the pO_2 probe has only to be connected to the MINIFOR and the MINIFOR has to be switched on.

Beware, **do not disconnect the pO_2 probe for more than a minute**, otherwise the **polarisation of the pO_2 probe will be lost**.

After sterilization and/or other activities, which creates a **disconnection of the pO_2 probe longer than 1 minute**, **do the polarisation again**.

In order to spare Polarisation time, it is advisable to keep the probe connected to the MINIFOR in the ‘Stand-by’ configuration.

The polarization time can be much shorter than 6 hours, it depends on its utilization. In case you have limited amount of time, then watch the signal after 1 hour of polarization and start with your work as soon as the signal remains stable for half an hour.

5.3.4 Oxygen saturation in water

Theoretic background information for the Calibration of pO_2.

Calculation of the saturation concentration of oxygen in water:

- ✓ Agitate and aerate medium in the vessel
- ✓ Read the temperature (after stabilization)
- ✓ Find out the altitude of the working place above sea level
- ✓ Get the relative air pressure (weather forecast) (If not, use the value 1013)

Use the following equation:

C = S x K x L

where,

- C = Calibration value
- S = Standard oxygen saturation value at the desired temperature, Refer the table below
- K = Altitude correction factor from the table below
- L = The ratio = Relative air pressure/1013

Example:

The calibration temperature = 18°C
Lab / working place is located at an altitude = 500 m
Atmospheric pressure = 1022hPa

Therefore,
S = 9.45 mg/l,
K = 0.943,
L = 1.0089

Calibration value = 8.99 or 9.0mg DO/l

Table 14 Oxygen saturation in water at various temperatures in mg DO/l at standard air pressure 1013 hPa (value S)

°C	mg O2/l	°C	mg O_2/l	°C	mg O_2/l	°C	mg O_2/l
0	14,64	10,5	11,12	21	8,90	31,5	7,36
0,5	14,43	11	10,99	21,5	8,82	32	7,30
1	14,23	11,5	10,87	22	8,73	32,5	7,24
1,5	14,03	12	10,75	22,5	8,65	33	7,18
2	13,83	12,5	10,63	23	8,57	33,5	7,12
2,5	13,64	13	10,51	23,5	8,49	34	7,06
3	13,45	13,5	10,39	24	8,41	34,5	7,00
3,5	13,27	14	10,28	24,5	8,33	35	6,94
4	13,09	14,5	10,17	25	8,25	35,5	6,89
4,5	12,92	15	10,06	25,5	8,18	36	6,83
5	12,75	15,5	9,95	26	8,11	36,5	6,78
5,5	12,58	16	9,85	26,5	8,03	37	6,72
6	12,42	16,5	9,74	27	7,96	37,5	6,67
6,5	12,26	17	9,64	27,5	7,89	38	6,61
7	12,11	17,5	9,54	28	7,82	38,5	6,56
7,5	11,96	18	9,45	28,5	7,75	39	6,51
8	11,81	18,5	9,35	29	7,69	39,5	6,46
8,5	11,67	19	9,26	29,5	7,62	40	6,41
9	11,53	19,5	9,17	30	7,55	40,5	6,36
9,5	11,39	20	9,08	30,5	7,49		
10	11,25	20,5	8,99	31	7,42		

Table 15 Correction for the elevation above sea level (value K)

elevation [m]	K	elevation [m]	K	elevation [m]	K	elevation [m]	K
0	1,000	360	0,959	720	0,919	1160	0,873
20	0,998	380	0,957	740	0,917	1200	0,869
40	0,995	400	0,954	760	0,915	1240	0,865
60	0,993	420	0,952	780	0,913	1280	0,861
80	0,991	440	0,950	800	0,911	1320	0,857
100	0,988	460	0,948	820	0,909	1360	0,853
120	0,986	480	0,946	840	0,907	1400	0,849
140	0,984	500	0,943	860	0,904	1440	0,845
160	0,981	520	0,941	880	0,902	1480	0,841
180	0,979	540	0,939	900	0,900	1520	0,837
200	0,977	560	0,937	920	0,898	1560	0,833
220	0,975	580	0,935	940	0,896	1600	0,830
240	0,972	600	0,932	960	0,894	1700	0,820
260	0,970	620	0,930	980	0,892	1800	0,810
280	0,968	640	0,928	1000	0,890	1900	0,801
300	0,966	660	0,926	1040	0,886	2000	0,792
320	0,963	680	0,924	1080	0,882		
340	0,961	700	0,922	1120	0,877		

5.3.5 Calibration of pO_2

The calibration of the pO_2 probe (DO probe, dissolved Oxygen Probe) is a two-point gauging with two standards:
A) Maximal pO_2 gauging (Slope point) and
B) Zero (0) gauging (Zero point)

In the dual point calibration, always start by the zero point calibration before calibrating the slope.

The tip of the probe should be placed about 1 cm from the edge of the closest stirring disc. This will assure a good exchange of the “saturated” liquid flowing to the membrane. At the same time, it will also help to displace air bubbles, which may form accidentally on the membrane.

For the 0.3L vessel: If you have mounted the butterfly-shaped stirrer disc instead of the micro-sparger, then make sure NOT TO USE any sparging!

If you use the butterfly-stirrer disc for minimal volume in the 0.3L vessel, then you have to make sure that no sparging takes place through the stirrer disc. Therefore clamp the tubing that leads to the stirrer axis with a tubing clamp. The maximal air saturation in minimal working will be reached, only by stirring – however, it will take more time.

Do not use the contaminated medium for the calibration of the maximal pO_2 value or for the control of maximal pO_2 value, since the contamination might consume the dissolved Oxygen and falsify your results.

For the calibration and crosschecking of the pO_2 calibration, for both the MINIFOR vessels with normal stirrer discs and butter-fly shaped stirrer discs, you can use any another vessel than the MINIFOR vessel.
Butter-fly shaped stirrer discs: Do not need sparging in vessel
Normal stirrer discs: Vessel should be saturated with gas
This might spare preparation time for the gas saturation of the liquid.
Make sure that not only the pO_2 probe but also the temperature probe (incorporated with the pH probe) present in the liquid.

The 'saturated' dissolved O_2 may vary according to the altitude (above sea level) of your laboratory / premises.

The step by step pO_2 calibration given is only an example. Try to follow these steps or choose a similar choice of the standards. But the steps should remain the same.

Calibration of Slope Point (Maximal pO_2):

Figure 5.3—1 After the polarization, the pO_2 probe has to be calibrated before and after sterilization. pH/Pt 100 probe should be inserted during calibration.

Figure 5.3—2 Make sure that the agitation value entered as SET value. Press the calibration key "C". (Please note that the agitation of the medium take place even in the calibration mode).

Figure 5.3—3 Move the cursor to the $[O_2]$ value using the right navigation key (>).

Figure 5.3—4 Check the ACTUAL value of the temperature (this should be the desired temperature for the run). Refer the corresponding Oxygen saturation value for the temperature in the table 5.3.4 Oxygen saturation in water.
[e.g. oxygen saturation in water at 26.5°C = 8.0 mg O_2/l]

Figure 5.3—5 Enter the value from the table in the SET [O_2] and then press the right arrow key (>). (e.g.: 80)

Figure 5.3—6 Once right arrow key is pressed, the value will be saved. (e.g.: 8.0)

Figure 5.3—7 Press the calibration key "C". The calibrated value will be shown as BUFFER 1 value of [O_2].

Calibration of Zero Point:

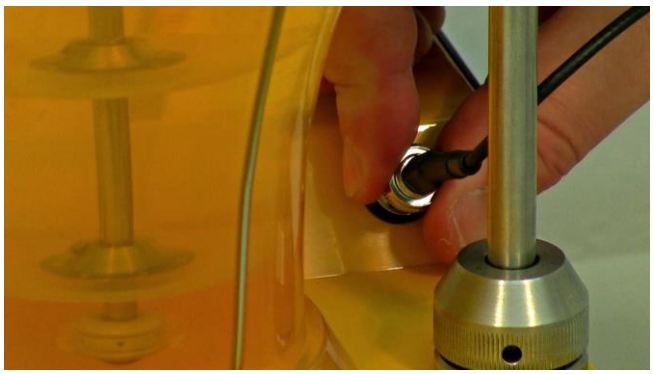


Figure 5.3—8 For the Zero point calibration, disconnect the pO_2 (DO) probe from the socket in base control unit.

Figure 5.3—9 DO probe should only be removed for not more than 1 minute as it lead to lose the polarization. If it is any longer than 1 minute, this will result in inaccurate readings.

Figure 5.3—10 The ACTUAL reading for dissolved O2 should always be '0' when the probe is disconnected. (If this is not the case then the probe may be faulty and you should check chapter Polarization, exchange of pO_2 Membrane / liquid, cleaning or contact the LAMBDA Helpdesk at support@lambda-instruments.com).

Figure 5.3—11 Enter the SET [O_2] value 00 using the number keys and press the right arrow key to save the value. The value will be displayed as 0.0 in the SET value.

Figure 5.3—12 Press the calibration key "C". The calibrated value will be saved and displayed as BUFFER 2 value of [O_2]. OK message will be displayed if the calibration done correctly.

Figure 5.3—13 Reconnect the pO2 (DO) probe to the MINIFOR base control unit.

Zero and Slope point calibration has to be repeated after sterilization. If not, it may affect the measurements.

5.3.6 Messages / Errors

MESSAGES which can occur

OK - calibration successful
ERR0 - same gauging solution used twice
ERR1 - same value entered twice

If you get an error message then you have to do the calibration again!

5.3.7 Crosscheck the pO_2 calibration

For crosschecking the calibration and testing the pO_2 probe, the following steps has to be done before and after sterilization and Polarization.

- ✓ Maximal (Slope) point: Air saturated medium used for calibration
- ✓ Minimal (Zero) point: 5% Na_2SO_3 solution

Checking and testing of the pO_2 probe with 5% Na_2SO_3 should be done after polarization and before calibrations and sterilization!

Crosschecking can be done in STANDBY MODE to save time.

Figure 5.3—14 Press the regultation key "R" to change the calibration mode to standby mode. Since the calibration is just complete, pO_2 probe will be still merged in the O_2-saturated medium / liquid.

Figure 5.3—15 The measurement of the O_2 saturated medium / liquid will be displayed in the ACTUAL Value of the parameter [O_2].
If it does not show the maximal value then make sure:
- Polarization was done.
- Cable is connected.
- The medium / liquid used is still air saturated.
- In case of using medium: check for contamination.

If nothing helps even after checking, the calibration has to be repeated.

Figure 5.3—16 Zero Point: It can be done only before sterilization (optional), using a 5% solution of Na_2SO_3. Immerse the probe in a 5% Na_2SO_3 solution and check for the ACTUAL value of the parameter [O_2] in the display.

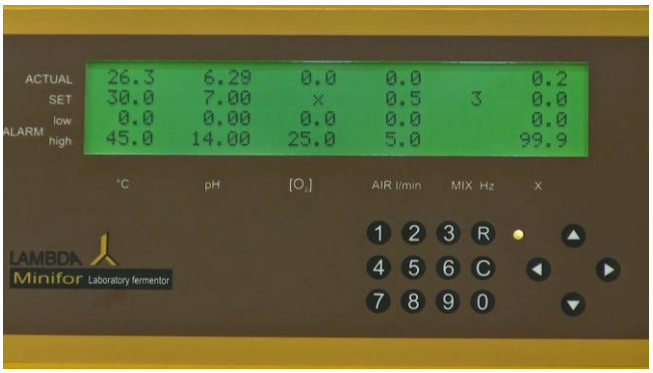


Figure 5.3—17 The 'ACTUAL' reading should show '0' after stabilization.

If the probe does not measure the minimal value = 0, then repeat the calibration of the pO_2 probe. If it has been repeated already, then refer the chapter 5.3.2

5.4 Pump flow calibration

If you need to know the amount of liquid added without a balance or if you want to measure the flow rate, then you will need the calibration of your LAMBDA pump speed / flow rate.
However, the inner diameter of the silicon tubing can change due to sterilization heat. The first sterilization has the biggest impact on the material. **Use only AUTOCLAVED tubing for calibration** and use the same tubing that you are going to use during fermentation.

The calibration of the pump flow rate with speed can be done to know the amount of the liquid added in the reaction vessel.

5.4.1 Volumetric Calibration

In the volumetric calibration, the amount of liquid pumped at a particular speed for a minute is calculated.

Figure 5.4—1 Connect the tubing to liquid in the bottle and set the desired speed (e.g. 600). Keep the measuring cylinder ready.

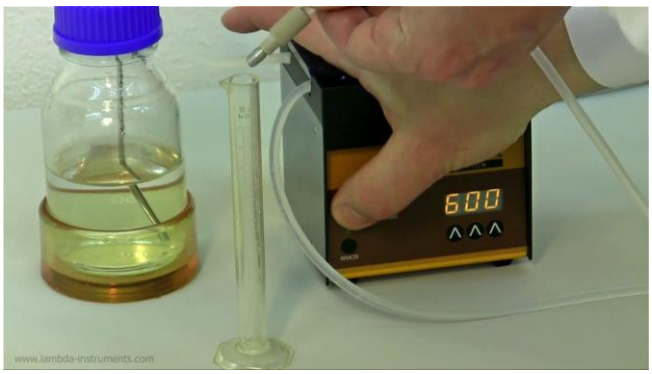


Figure 5.4—2 Turn the pump ON and have the other end of the tubing ready near the measuring cylinder. Carefully collect the liquid being pumped in the measuring cylinder for about 60 seconds.

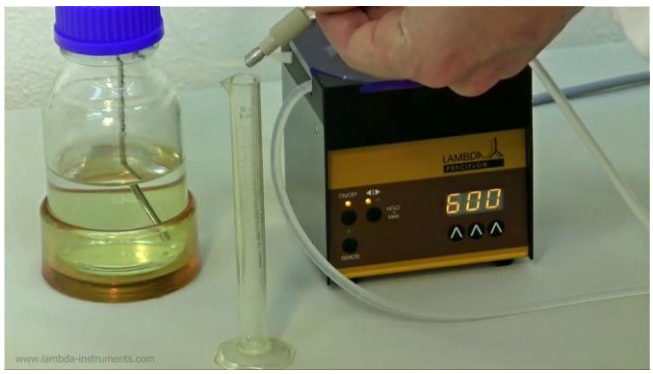


Figure 5.4—3 Collect the liquid in the measuring cylinder for about 60 seconds.

Figure 5.4—4 Exactly after 60 seconds, switch OFF the pump.

Figure 5.4—5 Measure the volume of liquid collected in a minute.
At the speed of 600, 3.2 ml/minute has been collected. Calculate the flow rate for other speed range using this value.

5.4.2 Calibration by weight

In the calibration by weight, the weight of the liquid pump in a minute is been calculated.

Figure 5.4—6 Measure the weight of the empty beaker using a sensitive weighing scale. For the exact measurement of the liquid collected, tare the weighing scale with the measuring beaker (Example: 0.000 g).

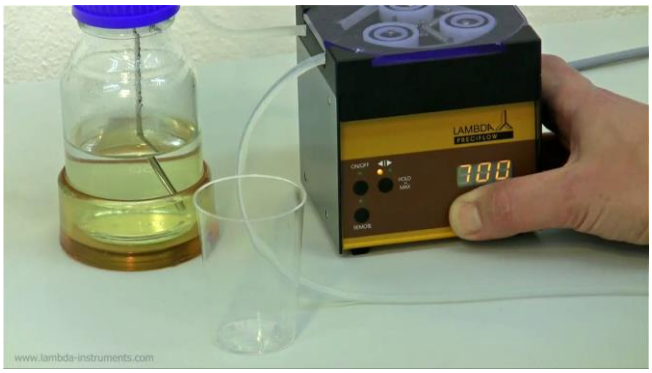


Figure 5.4—7 Connect the one end of the tubing with the liquid in the bottle. Set the speed of the pump say for example: 700.

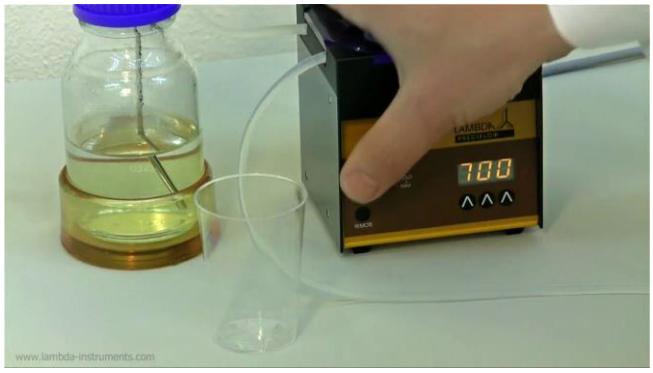


Figure 5.4—8 Switch the pump ON and have the other end of the tubing ready near the measuring beaker.

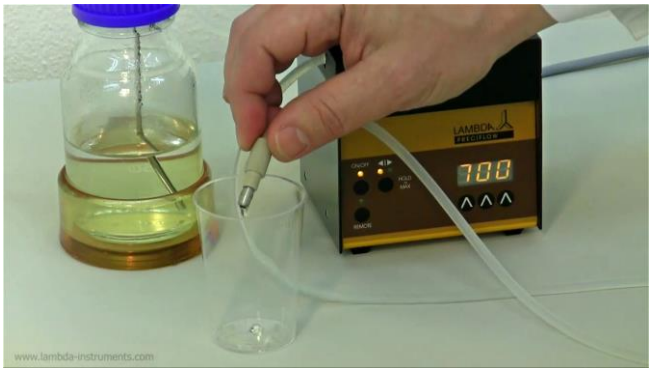


Figure 5.4—9 Carefully collect the liquid being pumped into the measuring beaker for about 60 seconds.

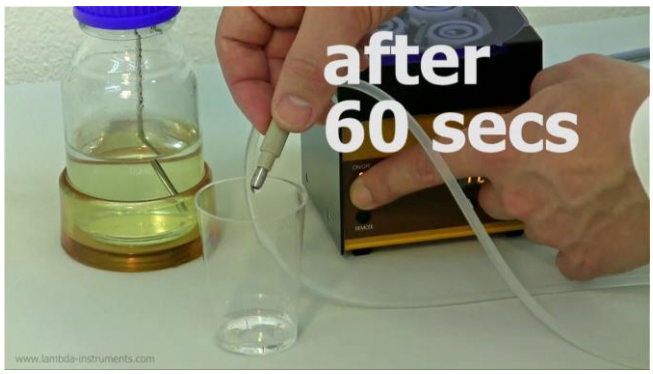


Figure 5.4—10 Exactly after 60 seconds, switch OFF the pump.

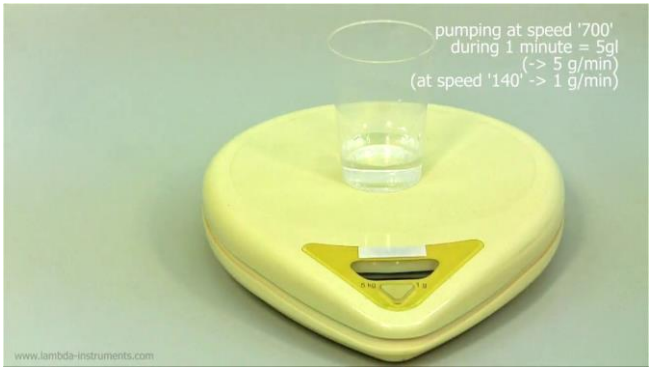


Figure 5.4—11 Weigh the measuring beaker with the liquid been collected in the past 60 seconds.

For example: At the pumping speed of 700, the weight of the liquid collected is 5 g/min. With this calculation, the weight of liquid collected at other pump speed can be found.

5.5 X-channel calibration (gauging): Example Weighing module

5.5.1 General introduction to the X-channel

"X" channel has the possibility to activate the "X" pump, above or below the preset value using the UP or DOWN option in the calibration of X-channel.

The level of acceptable "X" channel signal is from 0 to 10 V DC.

- If this signal is under 0 V (e.g. wrong polarity), an indication _ (underscore sign) will appear on the left of the actual value number of the parameter "X".
- If the voltage exceeds the 10 V, a sign **^** (Caret or up-arrow sign) will appear.
- There is no special indication when the signal is in the correct range 0 to 10 V.

The channel X can be used to control the scale module for continuous culture, conductivity and CO_2 probes.

For example: The weighing module allows the setting of a desired weight, which is kept constant. The "X" pump will be activated when the preset weight is exceeded.

5.5.2 Calibration of Weighing module

The scale module contains a sensitive sensor. Avoid any shocks of the sensor and handle it with great care.

Do not try to use the weighing module as a balance. The weighing module is just used for detecting the change of weight and not measuring the absolute mass.

Do not apply any force on the MINIFOR while using the weighing module – in particular on the front part where the weighing module is placed. Here, vertical forces will have an impact on the detected change of weight and the pump will be activated.

Calibration has to be done with the filled vessel. The calibration could be done by setting the starting weight to zero. However, it is more practical to set the initial weight to a certain value. In this way also negative weight (lower than zero) can be easily recognized.

Please refer the chapter 3.13.3 Range selection, before calibrating the weighing module.

The optimal positioning of the weighing tip on the scale module will be indicated by the yellow LED light. Once the scale module is well positioned, the calibration has to be initialized.

Figure 5.5—1 When the vessel is well positioned on the scale module, press the calibration key “C” to change the standby mode to the calibration mode.

Figure 5.5—2 Long press of the calibration key "C" will activate the SET values on the display.

Figure 5.5—3 Once the cursor is activated, use the right navigation key (>) to choose the parameter "X".

Figure 5.5—4 The calibration could be done by setting the starting weight to zero. However, it is more practical to set the initial weight to a certain value. In this way also negative weight (lower than zero) can be easily recognized.

Enter for example 100 in the SET value of the "X" parameter and press the right arrow key.

Figure 5.5—5 Entered 100 will appear as 10.0 in the SET display after pressing the right arrow key.

Figure 5.5—6 Place a known weight on the MINIFOR base control unit. For example, place an emply glass bottle.

Figure 5.5—7 Observe the actual value of the measured weight. When the reading becomes stable, wait until the message “HOLD” appears. And then press the calibration key "C" to calibrate the initial value.

Figure 5.5—8 Once calibrated, the initial value will appear as BUFFER 1 value of the parameter “X”.

Figure 5.5—9 Then place a known weight or add a certain known volume of liquid to the glass bottle. Say 100 g or ml.

Figure 5.5—10 Enter the final value to be detected and controlled by the scale module. For example: enter 200 and press the right arrow key (>) to save the value.

Figure 5.5—11 The entered 200 will appear as 20.0 in the SET value after pressing the right arrow key (>).

Figure 5.5—12 Wait until the message “HOLD” appears and then press the calibration key “C”. The message “OK” will appear indicating that the calibration has been accepted by the MINIFOR fermentor unit. Now, the scale is calibrated in g or ml. Weight calibrations can be made according to your need with weights.

Figure 5.5—13 Press the regulation key "R" to exit the calibration mode.

5.5.3 Setting up of the pump regulation

The weight can be controlled either by,

* **Addition (“UP”- mode**) or
* **Removal (“DOWN”- mode)** of medium solution.

This function allows selecting the sense of regulation of the “X” pump function:

It can either,
* **Switch on the pump “X” after the preset value is attained** or alternatively
* **Switch off the pump “X”**

Figure 5.5—14 To change the pump regulation, press the calibration key "C" to change the standby mode to calibration mode.

Figure 5.5—15 Press the right arrow navigation key (>) to select the SET value “UP” in the column before the parameter “X”.

Figure 5.5—16 Use the down navigation key to change to DOWN option.

Figure 5.5—17 Use the up arrow navigation key to change from DOWN to UP.

Figure 5.5—18 Press the right arrow navigation key to save the value, once the regulation of pump is selected.

Figure 5.5—19 Press the regulation key "R" to exit the calibration mode.

5.6 Antifoam system

For the best antifoam control, the ANTIFO and DOZITO needs to be calibrated accordingly:

- ✓ ANTIFO: Adjustment of the sensitivity of the foam detector
- ✓ DOZITO: Adjustment of the amount of antifoam added per time step

5.6.1 Sensitivity adjustment in ANTIFO

Each culture medium, depending on its salt content, produces a certain background conductivity. This conductivity will occur even without the formation of the foam and it should be taken into account by setting up a medium specific conductivity threshold.

Set and start the agitation optimized for the planned process condition and wait for about a minute, till a thin film of medium cover the inner walls of the reactor vessel.

For adjusting the sensitivity threshold for the given medium, rotate the knob clockwise on the ANTIFO until the light of the SET LED diode just switches from yellow to green.

Figure 5.6—1 Once the connections are made, the ANTIFO LED will be illuminated as green.

Figure 5.6—2 Rotate the ANTIFO knob clockwise gently to find a point where the green LED turns to yellow as shown.

Figure 5.6—3 Now rotate the knob anticlockwise (about 1mm) to set the sensitivity point and the LED displays green i.e. the point at which yellow turns to green.

Figure 5.6—4 Sensitivity of the ANTIFO for the particular culture is now set. The LED will turn from green to yellow, when it controls the DOZITO for antifoam addition.

5.6.2 Setting up of volume step in DOZITO

The excessive addition of the antifoam agent (anti-foam over-dosage) is detrimental for the oxygen transfer from the air bubbles into the culture medium and it is avoided by the waiting interval of about 20 seconds after the addition of each dose of the antifoam agent. The next anti-foam agent will be dosed only if the foam does not disappear during the elapsed waiting interval time.

Additionally, the **anti-foam dosage can also be controlled** by the volumetric addition step setting on the LAMBDA DOZITO miniature syringe pump.

The volume of added anti-foam agent can be varied from a dozen of micro-litres to about 0.3 ml.

The screwing for the volume settings on DOZITO must be done carefully; otherwise the end sensor may get damaged.

Never pull the pusher rod in the DOZTIO pump in the opposite direction!

Figure 5.6—5 Use the key given with the ANTIFO and DOZITO kit for setting up the volume of the anti-foam agent added.

Figure 5.6—6 Insert the key at the insert point next to the pusher rod.

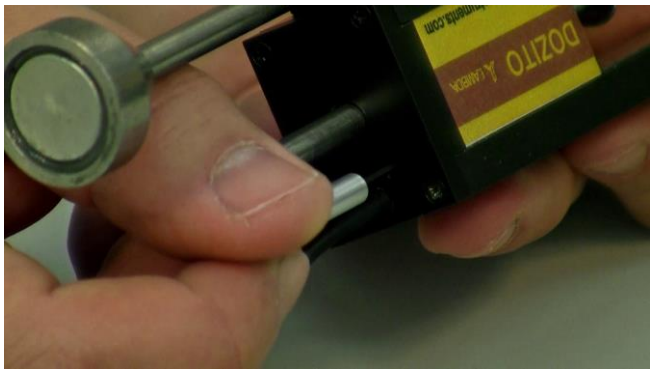


Figure 5.6—7 Screwing in = clockwise rotation = decrease of the delivered anti-foam volume / step

Figure 5.6—8 screwing out = counter-clockwise rotation = increase of the delivered antifoam volume / step

This volume adjustment screw is protected against being completely screwed out.

Operating state of the DOZITO Syringe pump (antifoam agent addition and waiting interval) can be depicted by the LED on the DOZITO.

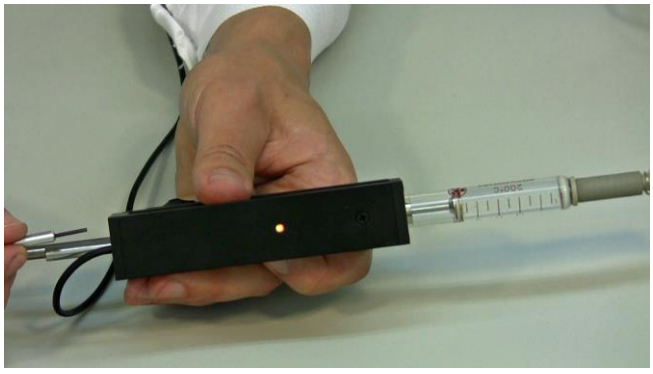


Figure 5.6—9 When the DOZITO receives the signal from ANTIFO, the LED will be illuminated as yellow and the pusher rod will push the syringe's plunger and thus delivers the pre-set volume of antifoam agent.

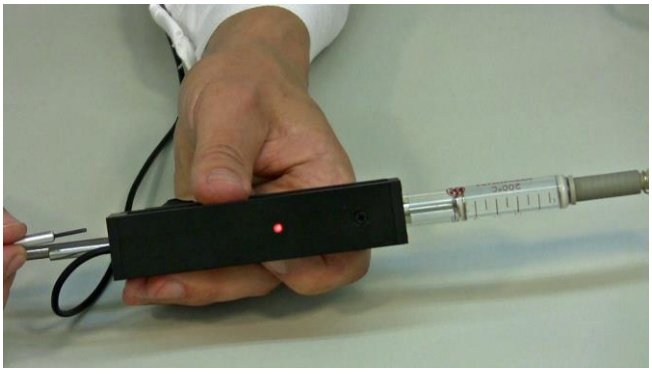


Figure 5.6—10 When the preset dose has been delivered, the LED will switch to red. Then the communication with ANTIFO is interrupted for approximately 20 seconds and the indicator LED is turned off.

6 PC Connection & Fermentation Software

Apart from the direct parameter control on the display, a process control system (PCS) for fully automatic control of the reaction and data storage is available.

Our experience shows that there is a big advantage to follow any culture and measure all the available parameters continuously. Cultures are very complex and a record of the parameters can show the reaction kinetics and for example, comparison of different experiments.

Work done with the MINIFOR fermentor-bioreactor is much more important and the results are much more valuable than the investment into a control system. MINIFOR can be used without an additional software, because all electronic control systems included in MINIFOR. And it is possible to upgrade the fermentation software at a later stage.

Fermentation softwares available:

- ✓ FNet easy-to-use fermentation software
- ✓ SIAM Industrial fermentation software
- ✓ MINI-4-gas automatic Gas-Mixing module software

6.1 FNet easy to use fermentation software

FNet is an easy to use software, which fulfils high demands on control and recording of most fermentations or cell cultures. Up to 6 fermentors can be controlled at the same time from one PC (preferable for normal laboratory cultures).

6.1.1 Ready to use software

- ➢ Easy to install and use.
- ➢ The software recognizes the connected fermenters at the start-up. Up to 6 fermenters, 12 Integrators and 6 Peristaltic pumps can be connected and controlled from one PC.
- ➢ No need to have the programming knowledge for installation and usage of the software.
- ➢ The cables are easy to connect and have standard connectors.
- ➢ No special add-in or license needed to buy for the connection of new fermenters and other instruments.

6.1.2 Data archiving

The process data (temperature, pH,...) are stored in a text file. This file can be exported to most of the programs on the market for the statistical analysis or reporting. More fermentation processes can then be compared for the process optimization.

6.1.3 Process Control

- Visualize the actual values and set values of temperature, pH, pO_2, air flow rate and an additional parameter X which could be for example the culture weight for continuous processes, optical density or an online biomass concentration measurement in a single window.
- Possibility to know the amount of base and acid added into the fermenter.
- Preprogrammed profiles on all the set points like temperature, pH, pO_2, air flow rate, agitation, parameter X or feed with a peristaltic pump can be done.
- Alarm management: The user can activate alarms for temperature, pH, pO_2, air flow rate, agitation, parameter X.
- The program also shows when an alarm has occurred.
- Different parameters can be observed and compared using the graph option with respect to time.

6.2 SIAM Industrial fermentation software

SIAM is a high quality industrial and laboratory software for professionals with almost unlimited possibilities for up to 99 fermentors and extended functions. It is user friendly and economic (low price and reduced learn phase). Developed specially for research laboratories, but it is also a tool for pilot plants and small production units. The user can build their own application within a few minutes.

6.2.1 Well Sophisticated Software

- Possibility to create a specific visual interface quickly and easily.
- Easy device (balances, controllers, etc...) connection and configuration.
- Real time display of parameters (like temperature, pH, etc...) evolution.
- The parameter values (analogue inputs for example) can be visualized and compared using graphs with respect to time.
- The user can choose the aspect for graph visualization:
    - The parameters to be displayed, the colour of the curve, the visualisation scale interval.
    - Up to 8 curves can be visualised on the same graph.
    - A zoom function is also available to reduce or to enhance the graph size. The user can read a particular value on the graph using the ruler.
    - Up to 4 ‘y’ axis can be visualized.
    - The channel values for the curves are stored permanently.
- Alert and Report Window:

- The report window allows creating a report about the automated process: alarms triggering, user intervening and remarks. The report content can be saved in a file and opened with a word processing software. With this window it is possible to trace the experiment (balance reset, controller or profile start-stop, etc...).

- Possibility to connect a large type of devices using a single software.
- Possibility to realize complex control strategies.
- Very good price / performance ratio.
- Exists in English, French, German

6.2.2 Devices analysed using SIAM

- MINFOR Fermentor-Bioreactors
- Peristaltic pumps: PRECIFLOW, MULTIFLOW, HiFLOW, MAXIFLOW
- Powder dosing system: DOSER
- INTEGRATOR
- Gas flow measurement system: MASSFLOW
- Syringe pump: VIT-FIT
- Interface cards from other manufacturers
- Balances
- Recorders and data loggers
- Temperature controllers, pH controllers, flow controllers, etc...

6.2.3 Applications

List of examples for the usage of SIAM in applications:

- Fermentor control in pharma industry (3 litres to 150 litres)
- Fermentor control in university research (300 ml to 300 litres)
- Measurement and control in climate chambers (temperature, humidity, light intensity, CO_2 concentration)
- Measurement and control in a wind channel (temperature, air speed)
- Measurement and control in pilot cheese dairy for research and development (vessel temperature, coagulation, press force, cellar)
- Measurement and control of chemical reactors, distillation installation in research and development
- Other applications in research and development, production or as education tool

6.3 MINI-4-gas automatic gas-mixing module software

The LAMBDA gas-mixing module allows a flexible mass flow controlled supply of different gasses with their individual gas flow paths. The gassing system automatically mixes up to four gasses for the cell culture system. The automatically controlled aeration system provides advanced gas diffusion by sparging and / or headspace gassing. The extension of the SIAM software allows a complete automatic gas-mix control for up to 4 MINIFOR parallel-reactors (4 x 4 gas flow controller).

6.3.1 Types of gas-mix systems

- 4-gas-mix for stem cell & mammalian cell culturing
- 3-gas-mix for anaerobe fermentation
- O_2-enrichment for microbial systems & biofuel development

6.3.2 Process Control

- Data storage & Plotting.
- Data acquisition, calculations & gas transfer rates.
- Trend graphs display.
- No software license issues for the addition of any further units and lab instruments.
- More than 4 MINIFOR units can be connected even if running without 4-gas control module.
- Helps to control the critical process parameters to optimize the growth rates as well as to achieve the highest titres during protein processing and hormone production.

6.4 PC Connection

The PC connection kit includes RS 232-485 converter, cables, USB connection, CD with program and an operation manual.

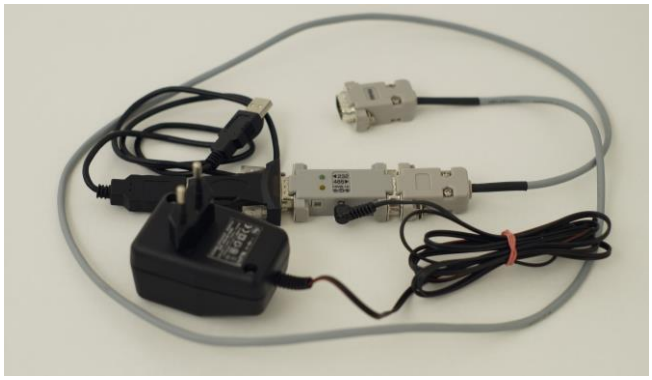


Figure 6.4—1 PC connection kit with RS-485 to RS-232 converter and USB connection.

Figure 6.4—2 Connect the PC connection cable to the DB 9 connector at the rear of the base control unit.

Figure 6.4—3 Connect the RS-485 to RS-232 converter to the other end of the PC connection cable.

Figure 6.4—4 Plug in the power supply for the RS-485 to RS-232 converter.

Figure 6.4—5 Illuminated LED indicates that the converter is ready to operate.

Figure 6.4—6 The PC connection cable along with the RS converter is connected to the PC with the help of the USB connector.

7 Sterilization & Reinstallation

Sterilization of the MINIFOR reaction vessel, storage bottles and tubings can be done in the autoclave and no in situ sterilization is needed.

Never add any cable into the autoclave, otherwise it will be destroyed!

Always fill the MINIFOR vessel before sterilization. But fill it with **not more than 2/3 of the vessel volume!**

7.1 Preparation for Sterilization

The reaction vessel with the parts that will be in contact with the sterile working medium, cell culture and the inner sterile gas flow has to be autoclaved to maintain the sterile working conditions.

Before Sterilization:

- ✓ The reaction vessel has to be filled with buffer or medium.
- ✓ In case of the 0.3L vessel, autoclave the vessel ONLY with liquid in the double jacket.
- ✓ The over-pressure valve should be inserted in the vessel.

- ✓ All unused side necks need to be fitted with a closed stopper, metal washer and a screw cap.
- ✓ The probe connectors have to be protected with a cap, unless they are of the VarioPin type, which does not need protection.
- ✓ All probes (pH, pO_2, X) which shall be used for the fermentation has to be calibrated.
- ✓ Make sure that all tubing which are connected to an end in the liquid medium are closed.
- ✓ The ventilation lines should be left open for Pressure compensation. If not, explosion or implosion may occur during autoclaving.

During sterilization, at least one set of tubing has to be used as **ventilation**. It means, at least the one **access to the fermentor head space has not to be clamped!** It is for compensation of possible over-pressure which may occur during the sterilization process.
In most cases, the **gas out-let is the best ventilation**, since the tubing has a big diameter. This should be **equipped with a gas filter for sterility** and the other end of the line should be in the head space of your MINIFOR vessel and not into the medium.

Check the gas filter on the line, which you use for the ventilation, before each sterilization! **An unclogged filter would prevent the vessel from bursting during pressure differences!**
To be sure that your gas filter is not clogged, test it by passing pressured gas through it. (e.g. by the gas line which you connected to the MINIFOR).

To reduce the risk of contamination, you can make all the needed connections with MINIFOR before sterilization and even sterilize the bottles with the correct solution. The tubings should be clamped to prevent the flow of the medium and liquids.

The **female cable connector without a pH probe should always be protected with a cap** or any other means against contamination! **Never leave it open.**

Figure 7.1—1 Fill 3/4th of the reaction vessel with buffer or medium.

Figure 7.1—2 Check whether the over-pressure valve has been inserted in the vessel.

Figure 7.1—3 Fit the closed stopper with metal washer and a screw cap on the unused side necks in the vessel.

Figure 7.1—4 Remove the grounding cable from the reaction vessel cannula.

Figure 7.1—5 Remove the agitator from the agitation unit. Carefully tilt the agitator back and forth for easy removal.

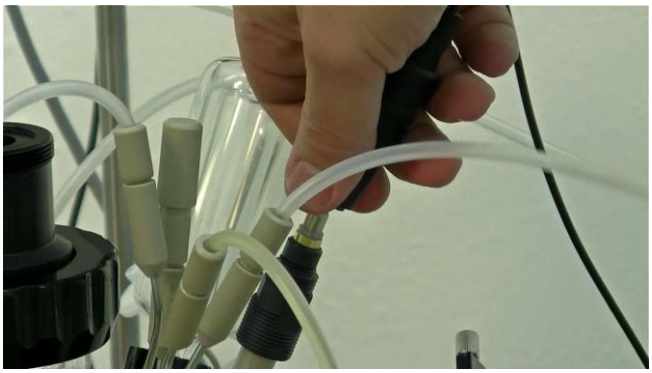


Figure 7.1—6 Disconnect the pO_2 probe connector cable from the pO_2 probe.

Figure 7.1—7 Disconnect the pH probe connector from the pH probe.

Figure 7.1—8 Protect the female cable connector using a cap against contamination.

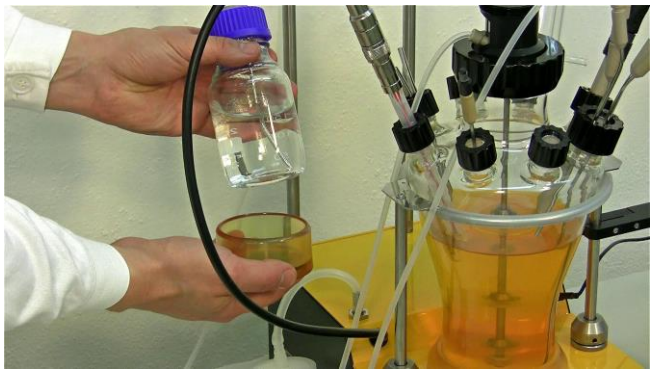


Figure 7.1—9 Remove the magnetic bottle holders from the storage bottles.

Figure 7.1—10 Store the magnetic bottle holders at the back of the main base control unit.

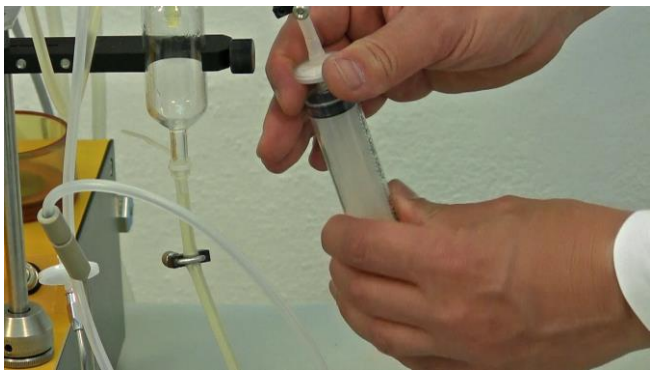


Figure 7.1—11 Remove the syringe from the sterile sampling device. Close all the tubing clamps.
If the sampling device is used then remove and disconnect it from the cannula and syringe.

Figure 7.1—12 Clamp all the tubings and air input line except the air output line with the out gas condenser and output filter.

Figure 7.1—13 Remove the acid and base tubing lines from the Peristaltic Pumps.

7.2 Sterilization of storage bottles and pump lines

Points to remember during the preparation of storage bottles and pump lines for sterilization:

- ✓ Remove the tubing lines from the Peristaltic pumps
- ✓ Fill the autoclavable, non-dangerous solutions into the bottle directly for sterilization.
- ✓ Fill the bottle **at a maximum of** 2/3rd with liquid / solutions.
- ✓ For safety reasons, it is highly recommended to autoclave the storage vessels with some drops of water instead of acid / base. After the sterilization, the sterile acid / base can be filled into the sterile storage vessels – applying the sterile handling rules.
- ✓ Clamp the tubing between the storage bottle and the fermentor vessel. The ventilation line (the line with gas filter on the bottle) has to be left open.

Figure 7.2—1 Storage bottle holders during sterilization.

Figure 7.2—2 Clap the tubing and check that the filter is not clogged. Place the bottles in the sterilization storage bottle holder.

Place the tubing clamp to the side of the bottle as close as possible. It prevents the stored liquid entering into the tubing during autoclaving.

Do not autoclave the corrosive or other dangerous agents in the storage bottles!
- Autoclave the storage bottle empty.
- Sterilize the dangerous and corrosive liquids separately.
- Add the corrosive / dangerous agents to the sterile storage bottle after sterilization by sterile handling.

While handling corrosives and other dangerous agents put on goggles and gloves and follow the safety instructions of your laboratory!

Do not use HCl as acid for your fermentation reaction as far as it is possible to use others like H_3PO_4 or H_2SO_4.

7.3 Sterilization of pH-temperature probe

No cable survives the conditions of sterilization. Each pH probe (and all other probes) must be disconnected from its cable during the sterilization in an autoclave.

The Variopin head (male connector) of the pH probe is constructed in a way that it can be sterilized in an autoclave without any covering cap. In case of contamination, it can be easily cleaned with distilled water and dried with a clean piece of paper towel.

The contaminated pH probe should not be connected to the probe connector cable! The **female cable connector without a pH probe should be always protected by a cap** or other means against contamination! Never leave it open on the bench.

If the pressure at the end of sterilization released too fast, the autoclave water may boil over and contaminate the connector of the pH probe. Since the autoclave water gets dirty frequently and it may contain salts, medium and other contaminants which would contaminate the pH probe. Thus, always **take great attention to clean the pH probe connector before inserting the probe into the connector cable**.

7.4 Checklist before sterilization

Since the MINIFOR Fermentor-Bioreactor system has varying sizes and holds distinct add-on according to the desired application, a general checklist has been given. This checklist can be adapted according to the system used.

Calibration:

- ✓ Calibrated pH probe
- ✓ Checked the pO_2 probe and pre-calibrated
- ✓ Pre-calibrated the 'X' Probe or Weighing module

Cables:

- ✓ Disconnected all the cables
- ✓ Protected cable heads from salty liquids and dust
- ✓ Protected the female cable connectors with protection cap

Reaction vessel:

- ✓ Mounted the MINIFOR reaction vessel in the vessel holder stand for autoclaving
- ✓ Filled 2/3rd of the MINIFOR with medium / buffer
- ✓ Inserted over-pressure valve
- ✓ Prepared access to inoculation (septum or bottle connection or welding or…)

Autoclavable gas filters:

- ✓ Gas inlet filter (leading into sparger tube and/or optional surface aeration)
- ✓ Gas Outlet filter (on the outlet gas condenser)

Storage bottles, tubings and clamps:

- ✓ Removed tubings out of the pumps
- ✓ Clamped the tubings that are connected with the liquid medium in the reaction vessel
- ✓ Placed the tubing clamp to the side of the bottle as close as possible
- ✓ The tubing without clamp should end in a line with an autocalvable gas filter for ventilation
- ✓ Empty storage bottles for corrosive / dangerous liquids are prepared with ventilation filter and tubing line
- ✓ Corrosive or dangerous liquids are filled with extra storage bottles for sterilization.
- ✓ In case of filtration of non-autoclavable liquids: empty storage bottles / lines to the fermentor vessel are prepared
- ✓ Removed magnetic holders from the storage bottles
- ✓ Mounted ventilation filter to the storage bottles
- ✓ Medium line for feed(s) and / or harvest is mounted (with storage bottles (empty or filled), bottle connection, welding tubing or …)

The signal of both pH and pO_2 probes is of very high impedance. Therefore any **dirt, salt solution or other contamination can negatively affect the precision** of the measurement. These contacts must be kept **clean**. (**Prevent over-boiling** at the end of sterilization).
The connectors on both pH and pO_2 probes can be cleaned with the distilled water and wiped off with clean paper towels.
The female plugs on cables cannot be cleaned and therefore, must be kept absolutely clean.

7.5 Autoclave space and Sterilization Cycle

Autoclave space for sterilization of MINIFOR Vessel					
Vessel type (L)	0.3	0.4	1	3	7
Mounted vessel dimention for autoclave: **Height H (cm)** **Diameter D (cm)**	 34 22	 22 23	 34 25	 37 34	 50 30
Amount of liquid / medium filled in the vessel for autoclaving	Fill water into the double jacket and close it using a silicone tubing.	Fill the maximum of 2/3rd of the vessel height.			

Sterilization time varies with the autoclave characteristics, temperature settings, vessel size and contents (i.e. media properties). If there is no specific requirement for sterilization, then start with the autoclaving conditions at 121 degrees Centigrade at a pressure of 15 to 20 psi with the of about holding time about 15 to 20 minutes.

Choose the autoclave **program for liquid sterilization** or any other program / manual handling with **slow cooling down phase / very good pressure compensation.**

Make sure that the **recorded temperature in the autoclave chamber is uniform** throughout the whole chamber.

Do not overload the autoclaving chamber. This may lead to a poor degree of sterilization.

Ensure that the **autoclave do not have any pressure leakage** as it will affect the sterilization process.

If possible sterilize all the tubing lines that are connected to the spare bottles and the storage bottles with the correction solutions.

7.6 Re-installation

After sterilization, check whether the tubing lines and connections stay secured and the filters dry.

If sterile-handling rules are fulfilled by the autoclaved items, then the re-installation of the MINIFOR has to be done. If not, the sterilization has to be done again.

Refer the chapter 3.15 Cable Connections for re-installation and cable connections.

7.7 Sterility test

Rule of thumb for sterility test:

- Batch fermentation with bacteria / yeast (up to 3 days) = short & cheap medium. Here the sterility test will cost more than it saves, both time and money.
- Continuous fermentation with mammalian cells = long & expensive medium. Here the sterility test will save a lot of time and money.

The sterility test has to be done with the same optimized conditions however without inoculation.

- Use the sterilized medium / buffer in the vessel and set the process parameters.
- Switch the MINIFOR to run mode and let it run without inoculation.
- Minimal duration of sterility test for the fermentation reaction of bacteria / yeast: between overnight and 24 hrs**.**
- The minimal duration of sterility test for culturing mammalian cells, plant cells and fungi: 2.5 days
- Check the sterility of the medium using the standard laboratory practices for checking the contamination.
    - If there is contamination, then the equipment has to be cleaned and sterilized again.
    - If not, the inoculation / seeding can be done.

If HACCP (Hazard management) should be made against a known contamination and if it is based on bacterial spores: set the best condition for that contamination during the next sterility test.
Example: Your growth phase at 37°C was perfect but you got Bacillus subtilis during your production phase at 30°C as contamination. Then you will do your next sterility test rather at 30°C to make sure that all spores were killed and the contamination will not again be detected later.

8 Inoculation

Before inoculating check the parameters needed for the culture and the necessary connections. Make sure that all measurement and control devices are connected and works well. Furthermore, the medium should be conditioned according to the growth phase specification of the culture.

If the needed working temperature is **lower than 4°C** or if you need cooling due to exothermic reaction, then you can shift the range of temperature control by the addition of the **optional Peltier cooling** device.

If you **do not need the automatic control of a particular parameter**, then include **0 as SET POINT** of that parameter

If you **do not need the acoustic and visual alert of a particular parameter**, then include **0.00 as a LOW ALARM** of that parameter

While working in the **humid surroundings** with **medium cooling**, **condensate** can be formed on the outer wall of the MINIFOR vessel and may displace downwards to reach the heating coil.
In order to prevent the condensate getting into the heating coil under the MINIFOR vessel, **place parafilm** around the vessel and vessel socket.

If you **doubt the temperature measurement** of the temperature probe, you can verify it by **mounting a thermometer** into the vessel and **comparing the measured values** of both sensors at the same time.

8.1 Checklist before inoculation

Since the MINIFOR Fermentor-Bioreactor system has varying sizes and holds distinct add-on according to the desired application, a general checklist has been given. This checklist can be adapted according to the system used.

If the sterility test is done in the same medium that will be used for inoculation, it would take some time for conditioning the medium for seeding / inoculation.

Probes:

- ✓ Connected the probes with the connector cable
- ✓ Probes or other devices should not touch the stirrer discs during agitation
- ✓ Polarization of pO_2 probe
- ✓ Re-calibration of pO_2 probe
- ✓ In case of using the weighting module: pre-calibrated the weighting module and everything put back in its best place? Is the X-pump disconnected when no continuous mode is needed?

Storage bottles, tubings and clamps:

- ✓ Filled liquids into the storage bottles
- ✓ Reinserted the tubings into the Pumps
- ✓ Connected the storage bottles to the MINIFOR vessel ports
- ✓ Removed clamps from tubings
- ✓ Removed clamps from gas tubing (sparger)
- ✓ Check all the tubings and clamps.

For MINIFOR 0.3L vessel:

- ✓ If butterfly-shaped stirrer disc for the 0.3L vessel is used: the air inlet tubing is clamped so that no sparging is allowed.

Air input:

- ✓ Connected pressurized gas to the rear of the MINIFOR base unit.

Parameter control:

- ✓ Set the parameters (set-points, alarm limits) according to the initial phase requirement of the experiment.

Septum adaptors and inoculum:

- ✓ Good access to septum or other device for inoculation / seeding
- ✓ Prepared the inoculum

When everything is as it should be, then switch the MINIFOR from standby to run / regulation mode by pressing **the R key**.

- ✓ Initialize the inoculation

If necessary, you can partially remove the cannula assembly from the quadruple port and each cannula needle, probes and other parts out of the vessel (e.g. for adjustment of their level in the vessel). There is practically no danger of contamination in this case.

Do not push the cannula needles further inside the vessel medium. The danger of contamination is considerable in this case. If this must be done, you have to use a convenient disinfectant. Traditional “flaming” or sterilization with hot air burners is also possible in some cases. This is reserved for connections on open vessels, but only when it is inevitable. The LAMBDA double-seal PEEK connections support temperatures of up to 300°C (and melt at 340°C).

8.2 Inoculation process

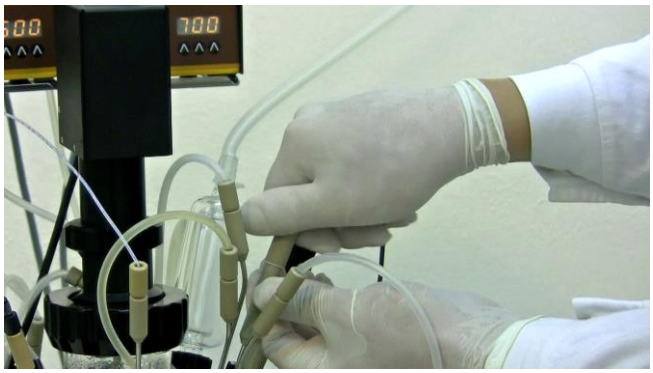


Figure 8.2—1 As a first step, loosen the septum protection cap.

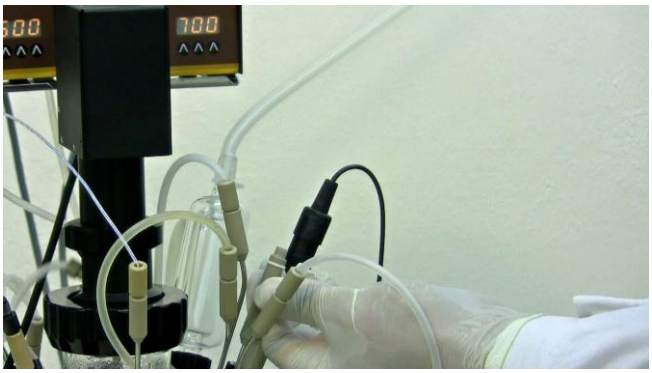


Figure 8.2—2 Remove the septum protection cap completely and get ready with the inoculum / inoculant.

Figure 8.2—3 Insert syringe loaded with the inoculant / inoculum along with the syringe needle into the septum adaptor body.

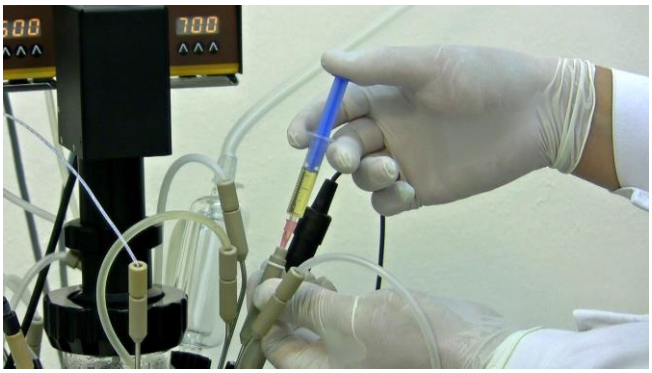


Figure 8.2—4 Inject the inoculum / inoculant into the liquid medium.

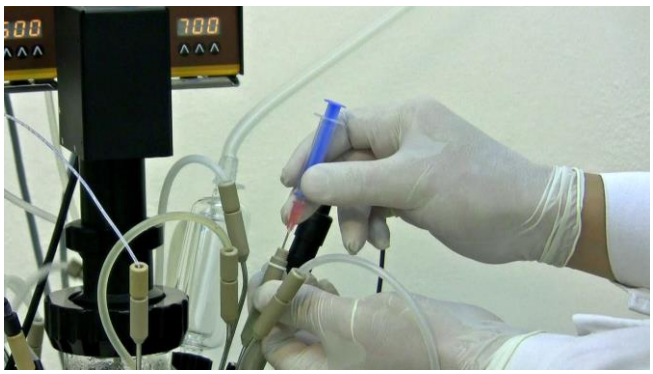


Figure 8.2—5 Remove the syringe along with its needle from the septum adaptor body.

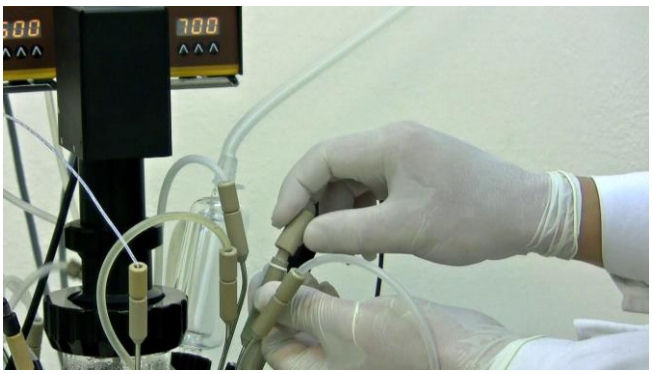


Figure 8.2—6 Replace the septum protection cap securely.

9 Sterile Sampling

Wash the line before each sampling to maintain the sterile conditions. The procedure is the same as that of taking the sample.

Never fill the sampling device completely, that the medium touches the inner part of the input tube! (If you need to take a high volume of sample, take it twice).

The sampling device has to be sterilized to maintain the sterile aseptic condition. The line has to be flushed before taking samples.

Washing the line:

Figure 8.2—1 Open the clamp on the tubing that connects the syringe.

Figure 8.2—2 Open the clamp on the sampling inlet (i.e. Between the MINIFOR and the sampling device)

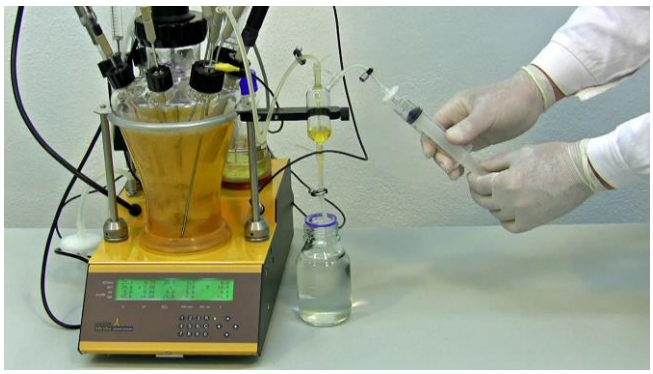


Figure 8.2—3 Pull the plunger of the syringe to fill the sampling glass device with the sample from the reaction vessel. Never fill the glass device completely.

Figure 8.2—4 Close the clamp on the inlet tubing to avoid the back flushing of the sample into the reaction vessel.

Figure 8.2—5 Remove the 70% Ethanol beaker / bottle into which the outlet tubing is dipped.

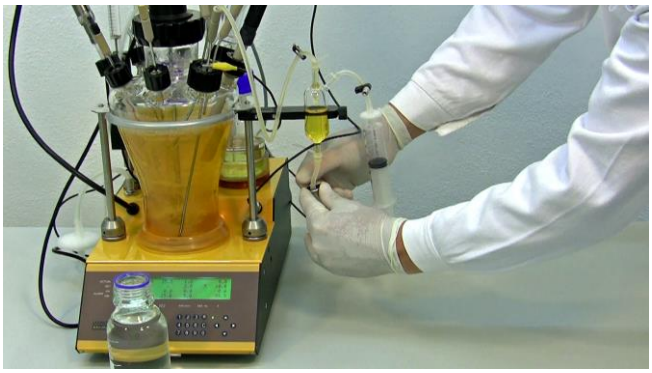


Figure 8.2—6 Open the clamp on the outlet tubing.

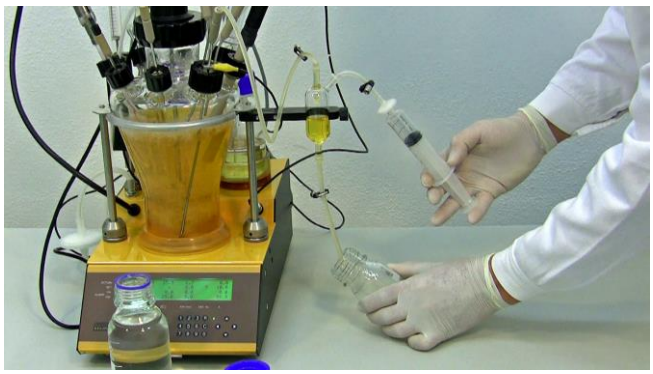


Figure 8.2—7 Use a beaker / bottle to collect the flushed waste sample. Push the syringe plunger to pass the air drawn into the sampling device and thus making the flow of the flushing liquid from the sampling device into the wastage bottle.

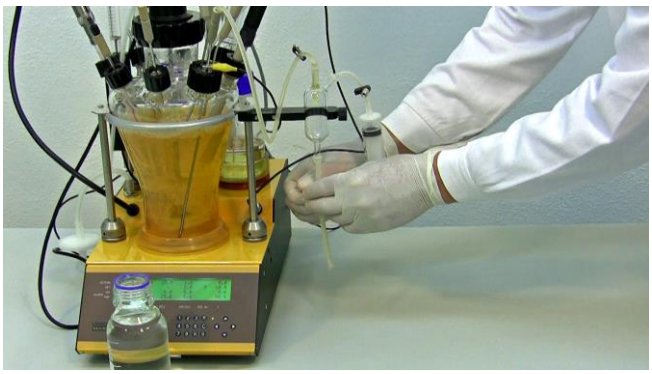


Figure 8.2—8 Close the clamp on the outlet tubing and if needed, place the outlet tubing in the 70% Ethanol solution even before taking sample.

Sampling:

After washing the sampling line, the sample can be taken into a sterile tube.

Figure 8.2—9 Open the clamp on the sampling inlet (i.e. Between the MINIFOR and the sampling device)

Figure 8.2—10 Pull the plunger of the syringe to fill the sampling glass device with the sample from the reaction vessel. Never fill the glass device completely. If the sample is needed in large amount, it can be sampled twice.
Close the clamp on the sampling inlet.
Open the clamp on the outlet.

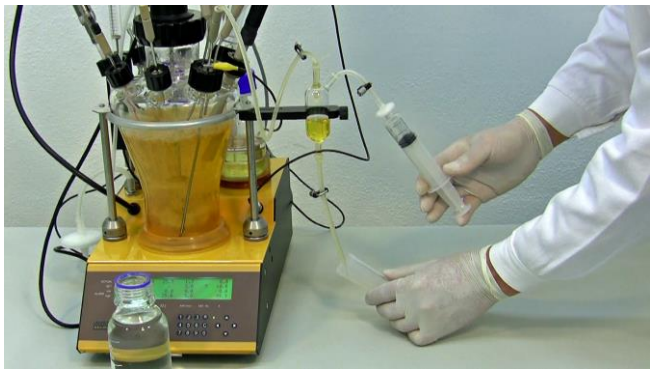


Figure 8.2—11 Push the syringe plunger to pass the air drawn into the sampling device and thus making the flow of the flushing liquid from the sampling device into the sterile sampling tube.
Secure the sampling tube with a cap.

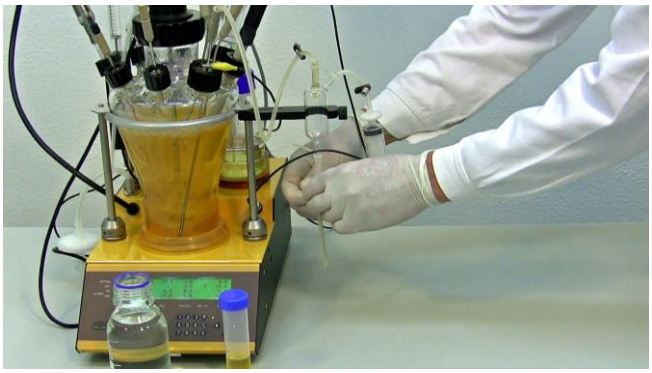


Figure 8.2—12 Close the clamp on the outlet tubing.

Figure 8.2—13 Once the sampling is done, place the outlet tubing into the 70% Ethanol solution and close all the clamps in the sampling device.

It is possible to push back the medium present in the sampling line to the reaction vessel. We normally **do not recommend** this, in case of contamination in the sampling trap, you may contaminate your reaction medium as well.

10 Maintenance

It is essential to clean and follow the preventive maintenance to keep the system in the proper working condition.

For removing the stoppers and probes, you can add a drop or two of distilled water between the stopper and the glass wall. Tilt them from side to side, while at the same time pulling them out.

Use brushes with mild detergent to remove the dirt and acidic solutions (acetic acid, citric acid or hydrochloric acid for salt deposits).

The elastic material of our microsparger does not require cleaning. If it should be blocked, the elastic body of the sparger will inflate and the blocking deposit will split off.

10.1 Overpressure security valve maintenance

The safety valve must be perfectly cleaned and the silicone tubing must be replaced after each overpressure event. Otherwise, the tubing may stick and prevent the correct operation of the over-pressure security valve

10.2 pH Probe maintenance and storage

All pH probes deliver an electric signal of very high impedance. This very faint signal can be affected by many external effects.

The sweat on fingers contains at least physiologic concentration of salts. If sweat contaminates the region of contacts of the glass and reference electrode it can drastically change the value of the measured pH. The same happens if contacts are contaminated by a buffer solution.

If a salty solution (or even distilled water or humidity) penetrates into the female connector of the pH probe connection cable it might make pH measurement virtually impossible. Unexplainable pH values may be observed when humidity and salts disturb the pH signal or even built-up electrochemical potentials. The measured pH signal might then even lead to a reading of pH 0 or 14, or stay constant around pH 7 due to a shortcut of the glass electrode signal.

In such a case, it is usually necessary to change the whole cable with the connector. It is therefore necessary to carry out absolutely clean work with the pH probe. LAMBDA and no other producer can change the laws of chemistry and physics so the probes must be treated with utmost care.

The Variopin head (male connector) of the pH probe is constructed so that it can be **sterilized in an autoclave without any covering cap**. In case of contamination, it can be easily cleaned with the distilled water and dried with a clean piece of paper towel.

In no case should a contaminated pH probe be pushed into the cable connector! **The female cable connector without a pH probe should be always protected by a cap** or other means against contamination! Never leave it open on the bench.

10.2.1 Cleaning pH probe

Clean the pH probe using distilled water and dry it with the blotting paper before storage.

In case of the contamination, the following procedures can be helpful to protect the electrode. However, these treatments may lead to the loss of our warranty.

Contamination by lipids:
Use detergents, solvents like ethanol, acetone, diethyl ether, strictly for a short time. Then wash it with distilled water.

Contamination with carbonate and metal hydroxides:
Stir the lower part of the probe in 10% HCl and wash with water.

Contamination with sulphides:
Stir the lower part of the probe in 10% HCl saturated with thiourea and wash with water.

Contamination by proteins:
Leave the probe in the 0.1M HCl containing 10 mg pepsin/ml for several hours and wash with water.

If nothing helps, you may try the old style cleaning with sulfochromic acid for 10 minutes and wash with distilled water in addition with KCl. Sulfochromic acid is very dangerous!

The **pH probe "does not like" long exposure to distilled water** and may become slow. The ion exchange zones in glass, necessary for the generation of the pH signal, may be perturbed.

10.2.2 pH probe storage

The probe should be stored standing upright, with the electrode tip immersed in a solution of 3 molar KCl or a buffer solution of a neutral pH range or normal tap water. (This keeps the ion exchange measurement zone of the glass equilibrated). If possible, do not dip the diaphragm into the liquid.

For storage longer than 10 days, it is better to wash the lower part of the probe with distilled water and keep it dry. Prevent contamination of the glass bulb with grease, organic solvents, strong acids and bases.

10.3 pO_2 probe maintenance and storage

The pO_2 probe can be cleaned using a soft tissue paper.

When the pO_2 probe shows a sluggish response, it may be due to the formation of deposits on the membrane. It is possible to clean the membrane with wet soft paper and a small amount of mild detergent. Finally, the membrane should be washed with the distilled water.

10.3.1 pO_2 membrane cleaning and maintenance

The pO_2 electrolyte is an alkaline solution (pH=13). Contact with skin, especially mucous tissues or eyes, should be avoided. Contact with the electrolyte is very likely during an exchange of electrolyte or the membrane module, the use of protective gloves is recommended. In case of contact, the area should be well washed with plenty of cold water. Get medical attention if adverse signs appear.

Great care should be exercised when handling the glass inner bodies, since any hairline cracks resulting from knocks, adversely affect sensor performance.

The DO probe membrane is very thin and therefore very sensitive to mechanical contact. Handle it very carefully.

When the DO probe begins to exhibit signs of failure (long response time, mechanical damage, increased residual current in oxygen free medium, etc.), it has to be replaced.

If the reaction is fast but the signal is unstable, it may be due to the perforation of the membrane. This also requires its replacement.

When replacing the membrane module and electrolyte, strictly observe the following instructions:

1. Hold the sensor in a vertical position (membrane downwards) and unscrew the old membrane.

2. Rinse the interior electrode body with distilled water and dry carefully with a soft lint free tissue. Dry the cathode (platinum circle diameter 1 mm at the tip of glass body) with a soft lint free tissue.

3. Visually inspect the O-ring for mechanical defects and replace if necessary.

4. Fill the membrane module approximately to the half with O2 electrolyte (about 30 drops of DO electrolyte (art. no. 800097: DO probe solution). Shake the electrolyte down in the tip direction and push the new membrane module on the slightly inclined body so that the silicone nipples inside the membrane module fit into the corresponding slots on the DO probe. Remove excess electrolyte with a tissue. Screw the membrane module sleeve onto the DO probe. The sleeve tightens easily over the O-ring.

5. After each exchange of the electrolyte or membrane module the DO sensor has to be re-polarized and re-calibrated (see the corresponding section of the LAMBDA MINIFOR manual).

10.4 Cleaning the reaction vessel

After each reaction, the vessel and the vessel components should be thoroughly cleaned. If necessary, decontaminate the vessel by autoclaving.

Always fill the MINIFOR vessel before autoclaving. But fill it with **not more than 2/3 of the vessel volume!**

Method 1:

Use a mild detergent and a test tube brush to clean the vessel and its side necks. Wash all the components in tap water. Rinse it with deionized or distilled water and dry thoroughly before placing it in the vessel holder.

Method 2:

Drain the vessel. Fill the vessel of about maximum working volume with water. Reassemble the vessel and its components and switch on the agitation. After 10-15 minutes, drain the water. Disassemble the vessel and its components, wash it with mild detergent.

10.5 Sterile inlet and exhaust filter maintenance

No cleaning is applied. Filter membranes that are blocked by moisture can be dried by gassing the filters carefully with pressurized dry and clean air. If foam or culture medium has entered the filters, they must be replaced.

The filters can be sterilized several times in the autoclave. However, the filters have to be replaced if damaged or blocked completely.

Clean the tubings used and carefully, check for the damage of the tubing. The tubing has to be replaced, if damaged.

11 Troubleshooting

When an error sign '_' appears under the stirrer values (mix Hz)

The alert and error sign means that there is water in the heating part (under the vessel) and therefore the safety system shuts down the heating.

Disconnect the Fermentor, and dry the heating system under the vessel and also the vessel bottom. Water can get into this area of the fermentor due to condensations as well. The best solution is to place a parafilm layer over the brim of the heating bowl – it isolates the heating coil from water.

How to supply pressurized air input?

Input air can be supplied to the fermentor using the AeroSilento (oil-free air compressor and vacuum pump).

Or gases from any air supply between 0.05 and 0.2 MPa. This can be from gas cylinders or pressurized air by the gas valves in your laboratories.

Fluctuation in pO_2 measurement

Bubbles, which may form accidentally on the membrane can cause a signal variation. Because of the fast response of the probe this can be seen as a signal variation, especially at low DO concentrations.

Long response time of pO_2 probe

If the response time of the DO probe takes longer, then it is a sign to exchange

- DO electrolyte
- Membrane module

When there is no air flow

The air input is on the rear side of the Minifor. From there the air passes through the MASSFLOW unit and then through the electronic needle valve. The air comes out of the opening on the left top of the base control unit. Please, do not use it in the opposite sequence.

When the culture is aerated, make sure about using a good quality air supply, which can deliver the air flow at the pressure range of 0.1 to 0.2 MPa, but not more.

Which processes need an automatic anti-foam control

Highly recommended to use the automatic antifoam control for the processes that include,

- Medium with BSA
- Formation of high protein concentration
- Fed-Batch with C-feed and high cell density of E.coli or yeast

For further questions, please contact support@lambda-instruments.com

12 Technical Specification

Compact microprocessor-controlled bench-top laboratory fermenter-bioreactor MINIFOR

Power	Universal power supply for mains 100-245 V AC/50-60Hz, 560W, CE conform
Display	LCD 4 x 40 digits with backlight illumination
Dimensions	22 x 40 x 38 cm (W x D x H)
Fermentor vessel	Pyrex borosilicate glass with 6 to 8 threaded necks; 0.3, 0.4, 1, 3, 6 litre vessels
Temperature control	High efficiency 150 W infrared (IR) radiation heat source with gilded parabolic reflector
Regulation	From 5°C over RT to 70°C
Measurement	From 0 to 99.9°C in 0.1°C steps
Precision	+/- 0.2°C (0 to 60°C)
Sensor	Pt 100 incorporated in the glass electrode of the pH probe
pH control	Sterilisable pH electrode pH 0-14 with automatic temperature correction, two-point semi-automatic calibration and Variopin connector
Resolution	0.01 pH unit
Precision	+/- 0.02 pH unit
pO_2 control	Sterilisable Clark type oxygen sensor with fast response, automatic temperature correction, two-point semiautomatic calibration, dissolved oxygen (DO) control through regulation of the airflow rate
Range	0 to 25 mg oxygen/ l, in 0.1 mg/l steps
Air flow	0 to 5 l/min in 0.01 l/min steps, measured by precise mass flow meter, linearity +/- 3%, reproducibility +/- 0.5%
Control	Proportional valve controlled by microprocessor
Supplied air pressure	0.05 – 0.2 MPa (0.5 - 2 atm)
Agitation	50 W Vibromixer 0 to 20 Hz (0 to 1200 rpm) in 0.1 Hz steps (6 rpm) with 1 or more stirring discs; Sterility similar to magnetic coupling
Selectable parameter	An additional parameter can be controlled by the instrument (foaming control, weight (for continuous cultures), pCO_2, redox potential, conductivity, optical density, etc.); with standard 0-10V or 0-20mA output
Ports	One large quadruple sampling or additions port with four needles with LAMBDA PEEK double-seal connections, used for sampling, inoculation, antifoam, feeds, harvest, addition of correction solutions etc., additional double ports are available
Pumps	Up to 4 independent pumps (PRECIFLOW,

	MULTIFLOW, HIFLOW or MAXIFLOW) with speed variation from 0 to 100 % can be used with MINIFOR laboratory fermenter-bioreactor
Gas flow control	In addition to pumps, several electronic flow controllers with flow rate ranges of 0-5 l/min (MASSFLOW 5000) or 0-500 ml/min (MASSFLOW 500) can be used for the controlled addition of gases (e.g. N_2, O_2, air, CO_2) in cell cultures; freely configurable gas station module
Working temperature	0 - 40 °C
Working humidity	0 - 90 % RH, not condensing
Security	IEC 1010/1
Weight	7.5 kg
PC control	Complete PC control and data processing using the fermentation software FNet (for up to 6 MINIFOR fermenters) or SIAM (for an even higher number of instruments)

13 CE Declaration

LAMBDA, hereby declares that the equipment described below conforms to the relevant fundamental safety and health requirements of the appropriate EC Directives, both in its basic design and construction as well as in the version marketed by us.

This declaration will cease to be valid if any modifications are made to the equipment without our express approval.

Product: Laboratory Fermenter - Bioreactor
Model: MINIFOR
Catalogue-No.: 800010

Corresponding EC directives:

- EC Low-Voltage Equipment Directive 73/23/EEC
amended by 93/68/EEC

- EC Directive on Electromagnetic Compatibility (EMC)
89/336 EEC

amended by 91/263/EEC; 92/31/EEC; 93/68/EEC

Harmonized standards applied:

EN 60950 (IEC 950:1991)
EN 50081-1, (EN 55022)
EN 50082-1, (EN 55024)
EN 61000-3-2 and EN 61000-3-3

The instrument was tested in a typical situation.

14 Warranty

LAMBDA provides a two-year guarantee on material and manufacturing defects, if the instrument was used according to the operation manual.

Conditions of guarantee:

- The instrument must be returned with a complete description of the defect or problem. In order to send back the equipment for repair, you will need a returns authorization number from LAMBDA.
- The customer will send the instrument to our service office.
- Damage or loss of items during transport will not be compensated for by LAMBDA.
- Failure to fulfill these requirements will disqualify the customer from compensation.

Serial Number: _____

Guarantee from: _____