



LAMBDA MINIFOR

Laboratory Fermenter-Bioreactor

OPERATION MANUAL



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LAMBDA MINIFOR laboratory fermentor-bioreactor

The MINIFOR was developed as a result of the need to construct a small laboratory fermenter for volumes from 0.035 to 5l. Based on long personal practical experience in fermentation we wanted to create a fermenter, which was easy to use and with the capacity to measure and control all the important parameters of the biological culture. The fermenter had to take up minimum space on the bench but with a good access to all parts. Several fermenters should, when placed side by side be suitable for the optimization of the parameters of growth of culture or optimisation of biotransformations etc. Each fermenter should be able to work independently or be connected to a PC for advanced regulation and extensive data treatment.

To keep the cost of the MINIFOR fermenter low without compromising quality several new ideas and innovations have been introduced:

Instead of a fermenter flask with a stainless steel cover, which is expensive, we use whole glass vessels with threaded fittings. They have been used for many years in cell culture and are proved to maintain perfect sterility.

Instead of a traditional propeller agitator, which requires an expensive motor and magnetic coupling, we have introduced a new vibration mixer. An electromagnet and an inexpensive membrane which can perfectly assure sterility and produce an efficient mixing without formation of a vortex (no baffles are needed). At the same time this type of mixing is gentler on cells and produces less foam.

The culture is heated by heat radiation produced in a parabolic radiator with a gold reflector placed under the fermentation vessel. The heat is adsorbed gently in the culture in a similar way to the sun heating water. There is no overheating of the culture, as is usually the case when a heater is placed in the medium and expensive double wall vessels with thermostatic baths are eliminated. At the same time pipes and cables disappear making the fermenter less complex.

As far as possible, expensive pieces of equipment have been replaced by new high performance plastics.

By using modern microprocessors it has been possible to position all the electronics in the front part of the instrument this makes the fermenter unbelievably compact and eliminates the casing tower usual in other products. Despite its small size six parameters are measured and controlled in the basic configuration of the MINIFOR.

LAMBDA Laboratory Instruments

is designer and producer of special laboratory instruments mainly for biotechnology and microbiology research and development.

LAMBDA MINIFOR - innovative and compact laboratory fermenter / bioreactor

LAMBDA OMNICOLL - fraction collector with new conception for unlimited number of fractions

LAMBDA PRECIFLOW, MULTIFLOW, HIFLOW and MAXIFLOW peristaltic pumps - practical, precise and very compact

LAMBDA SAFETY DOSER - allows automatic addition of powder without spoon. Safe operation with hazardous material (GLP)

LAMBDA VIT-FIT polyvalent syringe pump - programmable infusion and filling from micro syringes to large volume syringes of 150 ml without an adapter

LAMBDA MASSFLOW is precise gas flow measurement and control with recording option

LAMBDA PUMP-FLOW INTEGRATOR - with LAMBDA pumps and DOSER allows the visualization and recording of the transferred volume

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1. ADVICES AND WARNINGS FOR THE SUCCESSFUL USE OF THE LAMBDA MINIFOR LABORATORY FERMENTER-BIOREACTOR SYSTEM

1.1 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. Use brushes with detergent to remove dirt and acidic solutions (acetic acid, citric acid or hydrochloric acid for salt deposits). Protect the glass surface from scratches made by sand or other hard materials.

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

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

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.

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.

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 come up to this place and increases the protection of the output filter against clogging.

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 **jacketted 0.3L reactors** must be used only with the vessel jacket **completely filled** with water. The hose connectors of the jacket are closed e.g. with silicone tubings.

When using the small jacketted 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 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 the 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).

All glass components are predestinated 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 tubes for repairs. Broken vessels can also be sent to us. Please, contact us for further information.

1.2 Sterility

If necessary, you can partially pull cannulae or probes and other parts out of vessel, e.g. for adjustment of their level in the vessel. There is practically no danger of contamination in this case.

However, **do not push them inside the vessel**. The danger of contamination is considerable in this case. If this must be done, you have to use a convenient disinfectant (for example 70% ethanol or 10% hydrogen peroxide solution or any other suitable disinfectant). Traditional "flaming" or sterilization with hot air burners is also possible in some cases. This is reserved to 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).

1.3 Connection of additional LAMBDA pumps to the MINIFOR fermenter-bioreactor

All LAMBDA pumps can be connected to the MINIFOR. In this case they are connected to the central power supply of the fermenter by the pump connection cables (art. No. 4810) with round male eight pin connectors on both sides. This provides automatically also the RS-485 line connection to the system.

All LAMBDA pumps are polyvalent and can be used independently from the MINIFOR laboratory fermenter. In this case, they need a separate power supply (art. no. 4820 or 4821).

When connected to the MINIFOR system and its RS-485 line the **pumps and additional MASSFLOW gas flow controllers must not be powered by any external power supply**, because the used 12V voltage of the external power supply and the MINIFOR power supply will not be exactly the same and current may flow from one instrument to the another. This could generate problems and damage the instruments. If such a connection is inevitable a diode in the power supply line would be needed.

1.4 Various

The pO₂ probe has a slightly larger diameter than the pH probe. It is therefore important to use the **green open stopper for the pO₂ probe** and any open transparent stopper for the pH probe. Use the orange open stopper for the pO₂ probe on the 0.3 L vessel.

During setting up the stirring axis, pay attention that you **do not touch the tips of the pH or pO₂ probes**.

If possible, **connect the grounding cable** to the needle of the sampling port which is dipped into the medium. This will stabilize readings of both the pH and pO₂ probes. Both have high impedance amplifiers and probes can thus function as antennas and pick up all kinds of „electronic smog“ in your lab.

Never put any cable into the autoclave, otherwise it will be destroyed!

For safety reasons the MINIFOR laboratory bioreactor-fermenter is equipped with a **spill sensor** under the vessel. If any liquid penetrates into the gilded IR radiator the heating will be stopped and an alarm is switched on. Clean the spill with distilled water and wipe it dry with a soft cloth to prevent the scratching of the gilded layer. Then the spill alarm will switch-off.

Do not hesitate to contact LAMBDA if you have doubts or additional questions.

2. PRESETTING FERMENTER

2.1 First steps

The first steps consist of the setting of initial conditions and calibration of probes.

Connect the line cord at the back of the fermenter and plug it into the power line (100-230V/50-60Hz). Switch on the fermenter. The LED on the front panel is yellow. The display shows the last entered parameters.



Fig. 1 Front side of the fermenter with control panel and back side of the fermenter

CONTROL-PANEL

MINIFOR has three states:

- **Standby:** The Status-LED is yellow and all numbers on the display are still. This is the state of the fermenter after switching on. The display shows the last entered parameters. In this state all measurement readings are shown, but the regulation switched off and the number keys blocked. This is the state after switching on.
- **Operation:** The regulation is activated by pressing key "R" and the LED turns to green.
- **Calibration:** Key "C" starts calibration of pH, pO₂, parameter X or setting of address for the PC control (depending on the choice with the cursor). The LED is yellow and the regulation off. (Only preset stirring is activated to allow precise calibration of pO₂ probe).

Function keys:

"R"-Key: Switches between Standby and Operation mode (RUN with REGULATION) of the fermenter.

"C"-Key: Switches between Standby (no regulation, LED is yellow) and the Calibration mode. During calibration this key memorizes the calibration values.

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 it 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.

The cursor will be deactivated automatically if no manipulation of the number keys or arrows has been effectuated during 15 seconds.

Number keys:

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

Input modification:

Place the cursor on the desired parameter. Enter the value with the number keys. The values are entered without decimal point. For example, 9.00 is entered as 900. The left arrow allows correction of the actual value, the other arrows save the entered value.

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

2.2 Probe calibration (gauging pH-probe)

- In standby mode press key "C". Move cursor on the column of the parameter to calibrate. Enter the value of the **first gauging solution** (e.g. pH 4.00) in the second line (SET VALUE) and press right arrow. The value is displayed in the line below. The probe can now be immersed into the gauging solution. The probe is stirred gently until the measured value (ACTUAL) will stabilise. Then key "C" is pressed again.

- The value of the **second gauging solution** is entered as described above and the right arrow pressed. The probe is immersed into the gauging solution, gently stirring until the measured value will be stable. Then key "C" is pressed. An OK message is displayed if the calibration has been performed correctly. Calibration can be interrupted at any moment by pressing key "R". If the calibration is interrupted the values of the former calibration remain unchanged. (For checking purposes, the probes are immersed in the gauging solutions and the correct values must be displayed.)

It is important that the sequence as described above be observed exactly! If not, the calibration has not been done properly and has to be repeated.

By pressing key "R" the fermenter switches to the Standby mode, and a new pressing of key "R" sets the fermenter into the Operation mode.

MESSAGES

- OK - calibration successful
- err0 - same gauging solution used twice
- err1 - same value entered twice

See also section 10.2 for further information about the correct handling of the pH/temp probe.

2.3 Setting the temperature

Move the cursor by using arrows on the field for the temperature setting (SET °C, second row, first column). Enter the desired temperature (*the value shown on the first line and first column corresponds to the measured temperature and cannot be changed*).

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.

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.

2.4 Setting of other parameters

The remaining parameters are set analogically to the temperature.

Remarks:

- If the cursor leaves the field (arrows right, up or down) the modified value is automatically saved.
- 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.
- The alarm is deactivated if key "C" is pressed (calibration mode). (The alarm 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 alarm is activated for ex. 0.01)

- For the mixing function (MIX) only the desired value can be entered. The actual value corresponds automatically to the desired value, because the frequency of mixing is controlled precisely through the electronics and deviation thereof is not possible.

2.5 Regulation of pO₂

The concentration of dissolved oxygen (pO₂) is regulated by continuous (step-less) variation of airflow. This regulation is activated by entering a value for pO₂ into the field of the desired pO₂ value. The symbol "x" appears on the display at the location of the desired airflow to point out that the airflow is determined by pO₂ 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 pO₂.

2.6 Calibration of probes

Calibration of pH-probe

See Probe calibration (Chapter 2.2)

Calibration of pO₂-probe

The calibration is similar to calibration of the pH probe. For the 0%-level the gauging solution is a 5% aqueous solution of Na₂SO₃ (first gauging solution).

Instead of this it is also possible to simply disconnect the cable of the oxygen probe from its socket O₂ on the right side of the vessel. The current of the Clark amperometric probe will fall to zero, which simulates the signal level for zero value of oxygen concentration. This procedure is much faster.

For the 100% saturation level, oxygen-saturated water is used (second gauging solution). It is usually the medium, which is agitated during the calibration. The saturating oxygen concentration in mg of dissolved oxygen per one liter of water and at the corresponding temperature can be taken from the enclosed table (see section 10.6).

Calibration of the X-channel

See Probe calibration (Chapter 2.2)

In addition to all other channels, the X channel has the possibility to select if the X pump is activated above or under the preset value. The direction UP or DOWN is selected by pressing the upper or lower cursor arrow. The level of acceptable X signal is from 0 to 10 V DC. If this signal is under 0 V (for ex. wrong polarity) an indication _ will appear on the left of the actual value number of parameter X. If the voltage exceeds 10 V a sign ^ will appear aside. There is no special indication when the signal is in the correct range 0 to 10 V.

The channel X can be used for continuous cultures with scale module (option). This allows setting a desired weight, which is kept constant. The X pump is activated when the preset weight is exceeded.



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

2.7 START/STOP-Key “R”

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

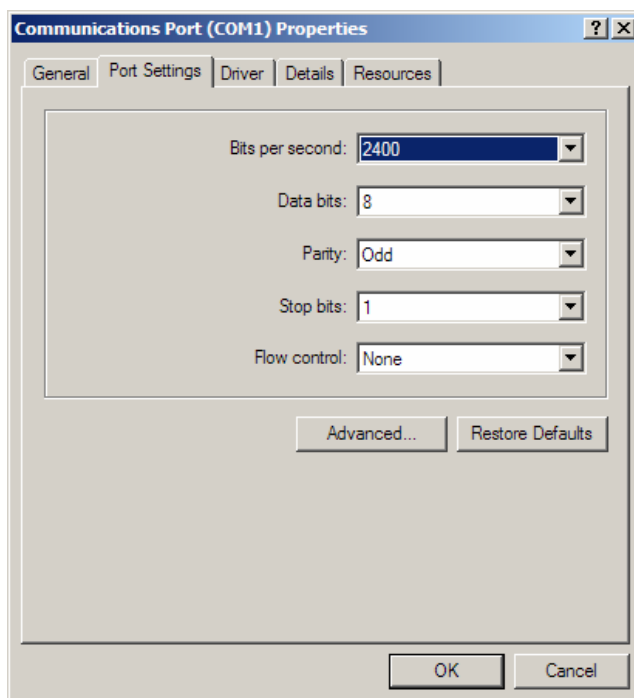
2.8 PC-Connection

The PC is connected at the back of the fermenter with a DB 9 connector. The different fermentation parameters can be modified from the PC. A Windows compatible program FNet or SIAM are available as option. It enables data acquisition and real-time visualization of the different parameters.

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

The laboratory fermenter LAMBDA MINIFOR is connected to the PC serial port through the RS-232/485 converter. Follow the steps indicated below:

- 1) Make sure the COM1 or COM2 of the PC is free and assigned to the serial port. Check the COM port settings (section “Ports (COM & LPT)” in the Windows® Device Manager):



In the “Advanced...” section uncheck the FIFO buffering option.

- 2) Switch the fermenter on and set the fermenter address to 01 (in the calibration mode of the fermenter)
- 3) Connect the fermenter to the COM1 or COM2 port
- 4) Install the software (during the installation the software automatically checks the presence of a device on COM1 or COM2)

3. PREPARATION AND STERILISATION OF VESSEL

3.1 Preparation of vessel

3.1.1 Mounting of the agitation/mixing system

Please see also section 10.11 for a step-by-step description of the eccentric stirring assembly. For the largest 7L vessel assembly see also section 10.13 for details on the use of the LAMBDA liquid O-ring.

The disassembled head is shown in Fig. 2.

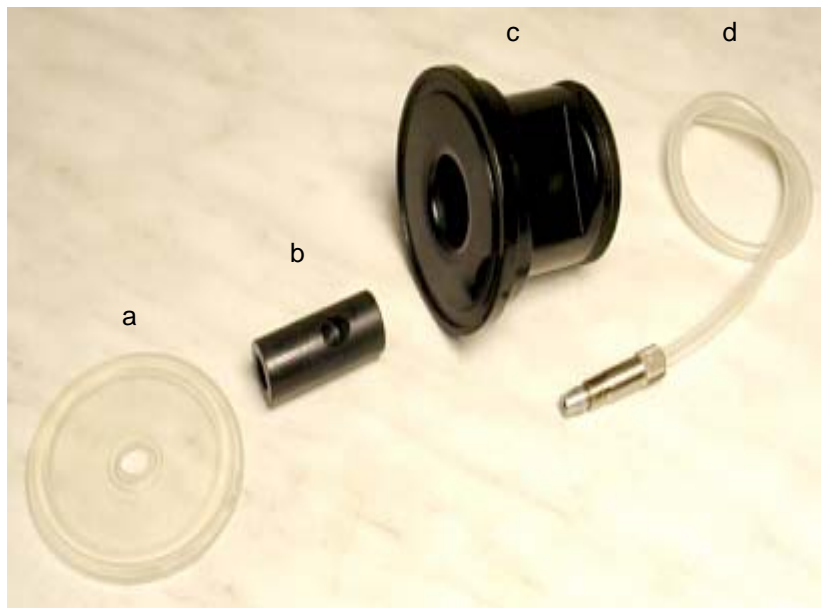


Fig. 2 Disassembled head of the vibration mixer

Slide the mobile part of the cock head 2b into the corpus of the cock head 2c (Fig. 3).

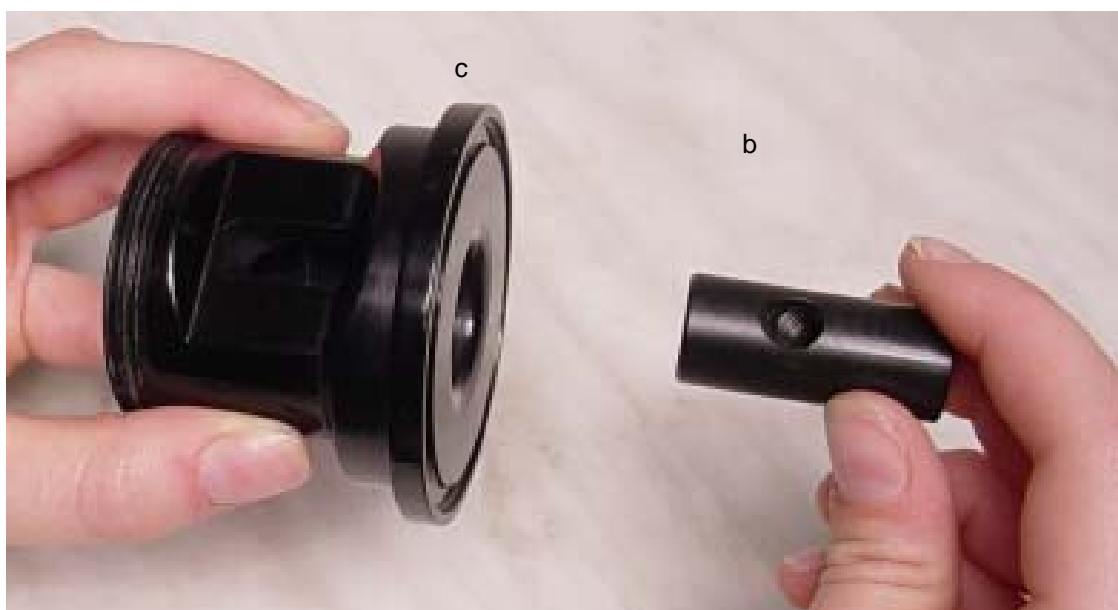


Fig. 3 Assembly of the cock head

3.1.2 Air inflow

A silicone tubing of 5mm outer diameter and 1mm wall thickness is used for the air inflow. The tubing is put through the tubular jacket 5f. A double-conical insert 4e is placed into the tube end (Fig. 4). It is easier if the tubing is made wet with distilled water or if you push it inside the table.



Fig. 4 A double-conical insert is placed into the tubing end

This assembly is screwed under inclination of 60° deeply into the mobile part of the cock head (Fig. 5). **Please pay attention to the right introduction into the thread and do not force the thread.**



Fig. 5 The tubing is screwed into the cock head

The screwing should go easily if the insertion is right! The tube is fixed to the corresponding filter (Fig. 6).



Fig. 6 The tubing is fixed to the corresponding filter.

Place the membrane 2a on the lower side of the cock head and screw the axis of the mixer on the mobile part of the cock head (Fig. 7).



This membrane guarantees the sterility and must be faultless and well mounted!



Fig. 7 Screwing the axis on the mobile part with membrane on the lower side of the cock head

The mixing disc is positioned on the axis, about 1 cm from the end, and tightened by hexagonal wrench (Fig. 8). If more stirring discs are used, place them in a distance of 4 to 6 cm on the stirring axis. The self-cleaning gas microsparger is fixed into the lower part of axis with a screw (Fig. 9).

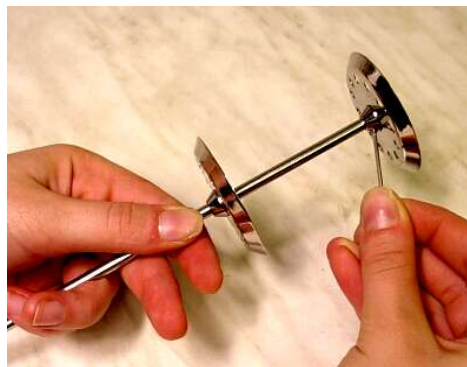


Fig. 8 The mixing disc is positioned on the axis

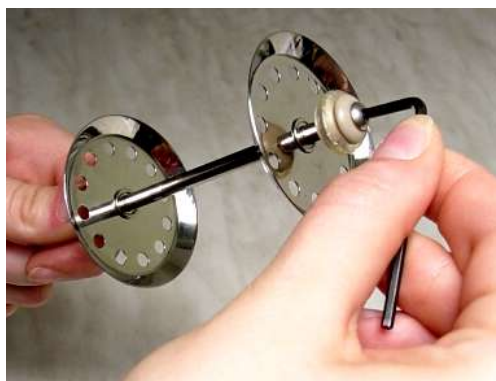


Fig. 9 The self-cleaning gas microsparger



The elastic material of our microsparger does not require any cleaning. If it should be blocked, the body of the sparger will inflate and the blocking deposit will split off. The tubing can be placed into the pump head easily after the sterilization.

The head is tightly screwed onto the fermenter vessel with the large nut 10g (Fig. 10).



Fig. 10 The fermenter vessel with the head

For mounting, it is advantageous to fix the fermenter vessel onto the housing (Fig. 11). The lateral holders are adjusted to the right height of the vessel 11h and fixed in position by rotating ring 11i. Elastic 11j O-rings affix the side-necks to the holder.



Fig. 11 Fixing the fermenter vessel onto the housing

3.1.3 Air outflow

Silicone seal, washer and screw-hole cap are placed onto the glass air outflow cooler (Fig. 12). A silicone tube is fixed onto the outflow cooler on one side and onto the corresponding filter on the other side. The air outflow cooler is now screwed into the side neck of the fermenter vessel (Fig. 13).



Fig. 12 Fixing of the glass air outflow cooler



Fig. 13 The vent-filter is placed on the cooler

Alternatively, the optional electro-thermic Peltier out-gas condenser can be used. It allows a much better condensation of water vapour, because of low temperature attained (about 5°C) and no cooling water is required.

For more information about the setting-up of the electrical Peltier out-gas condenser see chapter 10.12.



In practice, the air filter is sometimes omitted and the out flowing air is lead into a 70% ethanol solution. Where the budget is low, this can spare money for the replacement of the output filter.

3.2 Ports for additions and sample collection

The large silicone plug is screwed, together with a washer, into the large side neck using the screw-hole cap Pyrex 30. (The insertion can be easier if the stopper is made wet with distilled water). Proprietary **LAMBDA PEEK DOUBLE LOCK** end pieces ^{*)} are mounted onto the top of four tubes (Fig. 14). The long tube (canula) reaching to the bottom of the vessel is preferably used for sample collection; shorter tubes are used for addition of pH-adjusting substances, inoculation and feeding. Alternatively, they can be used as the upper contact for foam detection or for adjustment of maximal medium level in continuous fermentations.

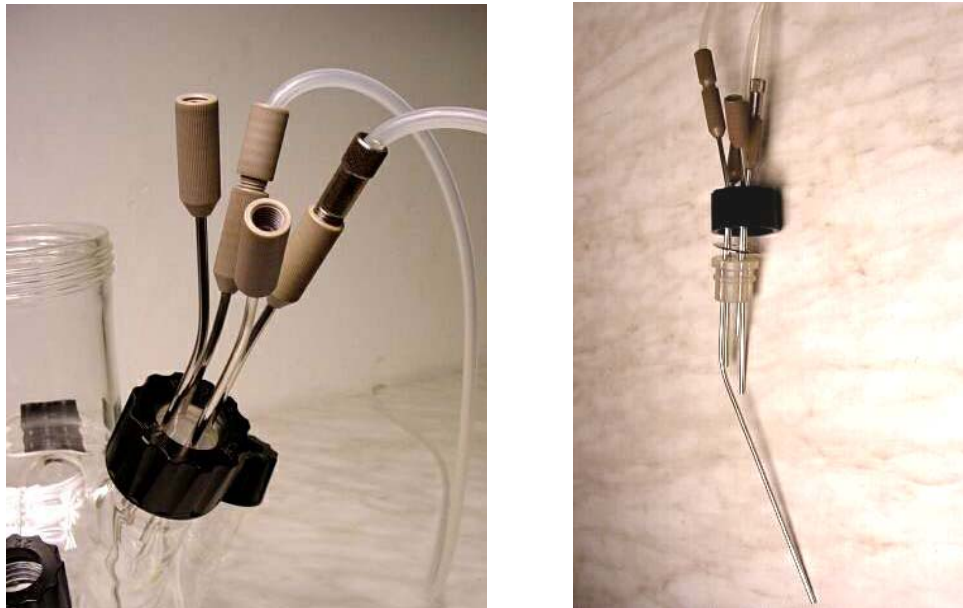


Fig. 14 Ports for additions and sample collection



To reduce the risk of contamination, we recommend to make all connections before sterilisation and sterilize bottles with correction solutions connected to the vessel. The tubings are clamped to prevent the flowing of the solutions into the vessel during sterilization. The tubing can be placed into the pump head easily after the sterilization.



^{*)} The LAMBDA DOUBLE LOCK connection system allows safe and easy connection of tubing to the vessel. It is made of PEEK. PEEK is 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 much higher mechanical stability and does not “flow” like PTFE. For its superior qualities, it has been selected by LAMBDA despite of its very high price.

3.3 Probes

The pH-, pO₂- or other X channel probes are calibrated. White open silicone plug is inserted into the side neck for pH probe (on the left side of the vessel) and **green open plug** to the side neck **for the pO₂ probe** (on the right side of the vessel). Place a metal washer on the silicone plug and loosely screw the screw cap. Push the probe into the stopper and secure it in right position by screw cap (Fig. 15).



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

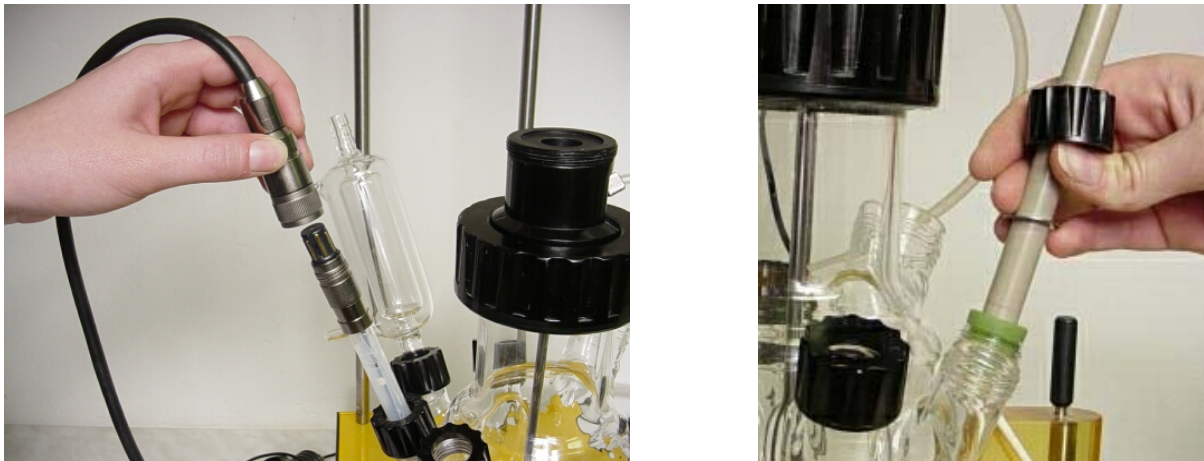


Fig. 15 Position of the pH-probe and the pO₂ probe (green plug)

The signal of both pH and pO₂ 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 contacts on both pH and pO₂ probes can be cleaned with distilled water and wiped off by clean paper towels.

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



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

Before sterilisation, the vessel is filled with medium, the over-pressure safety valve is added on one of the ports (see section 10.1) and all unused side necks are then fitted with closed stopper, metal washer and a screw cap. The probe connectors have also to be protected with a cap, unless they are of the VarioPin type, which does not need protection.

3.4 Sterilisation

The fermenter vessel is sterilised as usual (e.g. 30 minutes at 120°C) and then cooled off. Generally, acid, base and the other solutions are sterilized in Pyrex flasks, together with the degassing filters, tubes etc., simultaneously with the fermenter vessel. All these elements have then to be protected from contamination as customary.

ADVICES:

- If possible sterilize all tubing lines connected to spare and correction solution containing bottles.
- Tubing should be clamped to prevent any transfer of solution into the fermenter vessel during sterilization.
- Vessel and bottles must have their sterile filters for the equalization of pressure fitted on.
- Be aware that any addition and any transfer of liquids and gases carries a risk of contamination and should be done with due precaution.
- The LAMBDA DOUBLE LOCK PEEK connectors can be exposed to temperatures up to 300°C and can be flamed with precaution with alcohol burners for a short time. Silicone tubing can tolerate temperatures up to 250°C. If required, metal connectors may be also supplied as an option.
- Never put the magnetic bottle holders into autoclave for sterilization they will be destroyed!

4. START OF THE FERMENTATION

4.1 Connections

The cooled off fermenter vessel is placed into the ring above the infrared heat emitter and has to be well supported by the lateral holders (Fig. 11). The lateral holders are fixed in position by rotating ring. It can be tightened strongly using a supplied rod. The tightening rod can be conveniently kept in the hole in the upper part of the support. Elastic O-rings affix the side-necks to the holder.

- Connect the compressed air of 0.1 MPa (**maximum 0.2 MPa!**) at the connector on the backside. The sterilised air inflow tubing containing the filter is fitted on the air tube at the front on the left. The tubing may be fixed by common clamps.
- The electromagnet 16k is screwed, as far as it will go, onto the axis of the cock (Fig. 16). The cap nut 17l is tightly screwed onto the cock head (Fig. 17).



Fig. 16 Screwing of electromagnet



Fig. 17 Screwing of the cap nut

- The probes are connected properly to the control unit by cables.
- The sterilised tubing is inserted into the pump heads, the pumps placed onto the racks and connected to the control unit of the fermenter. The tubings are connected to the supply bottles (when it has not already been done before sterilization). The tubing ends are preferably passed by flame to avoid any contamination.
- The supply bottles are placed into the magnetic supports and positioned onto the housing of the fermenter (Fig. 18). Connecting tubing is not shown here for simplicity.



Fig. 18 Position of supply bottles

- Switch on the pumps (connected to corresponding sockets on the rear of fermenter by cables), press REMOTE key and choose the direction of rotation. They are still blocked by the microprocessor of the MINIFOR until the “R” RUN button is not pressed to start up the run.
- Cooling loop can be inserted through one of smaller side necks of the vessel. The flow and temperature of the cooling liquid circulated through the loop is adjusted to provide excessive heat removal. The temperature is regulated by the increased power of the radiation heater. In this way, the high precision of the preset temperature is maintained.

4.2 Start-up of the fermenter

Set all values of parameters to required values.

After a last control of all connections, press key **R**. Regulation is now activated.

Once the desired parameter values are reached, inoculation can be performed.

5. PRACTICAL HINTS

- Due to the narrowness of the vessel, it is important that it be supported adequately during sterilisation.
- Use magnetic supports for the supply bottles. The vibrations resulting from the mixer could otherwise start moving them.
- To avoid excessive heating of the airflow regulation valve, do not activate the regulation (LED green) when the fermenter has not been connected to the compressed air.



The airflow is used to cool down the airflow regulation gate. If there is no air on the input, the valve opens maximally and since the filament is not cooled by streaming air it can overheat. To avoid damage, the valve will be automatically switched off after a while and the preset value of the air regulator will be set to zero. As a result of overheating it may take time for the valve to close down completely the air flow.

- The LAMBDA MINIFOR bench-top laboratory fermenter-bioreactor does not need any special maintenance. Keep the fermenter clean. Clean it with a humid cloth. Common detergents or ethanol can be used as well.
- **Resetting to original state:** To prevent blocking of the system by wrong series of manipulation by client, the reset function to the original state has been added to the system.
 - Enter the MINIFOR calibration state
 - Write Adr 99
 - Press the right arrow
 - Press C button
 - Switch off the MINIFOR
 - Switch the MINIFOR on again

6. SAFETY PRECAUTIONS

If a liquid or saline solution gets into the back side of the fermenter (power supply), pull out the line cord **immediately** and contact our customer service.

7. TECHNICAL SPECIFICATIONS

Compact microprocessor-controlled bench-top laboratory fermenter-bioreactor MINIFOR

Display:	backlit LCD 4x 40 characters
Power supply:	power line ~100-245 V /50-60 Hz, 400 W, CE conform
Operating temperature:	0 - 40 °C
Relative humidity:	0 - 90 %
Security:	IEC 1010/1
Weight:	7.5 kg
Dimension:	22 x 38 x 40 cm (B x H x T) (mounted)
Working volume:	from 35 ml to over 5 l
Vessel:	Pyrex borosilicate glass, 6 or 9 threaded necks
Mixing:	Vibromixer 40W, 0-20 Hz (corresponding to 0-1200 rpm)

Temperature Control:	Infrared radiator 150 W with gold reflector, operating from RT +5°C to 60°C
Resolution:	0.1°C
Precision:	+/- 0.2°C (0 – 60°C)
Thermo sensing:	high precision Pt 100 built in the glass electrode bulb for fast response
pH-Control:	combined sterilisable glass electrode for pH 0-13 with electrolyte in nano-suspension, VarioPin connector, two point calibration
Precision:	+/- 0.02 pH
Resolution:	0.01 pH, automatic temperature compensation
Pump:	high quality pumps PRECIFLOW, MULTIFLOW, HIFLOW, MAXIFLOW flow rates 0-10 l/hr
Pump connection:	up to 4 pumps with progressive proportional flow rate control
O ₂ -Measurement and regulation:	0 to 25 mg/ml, sterilisable Clark-probe all PEEK body with large protected glass reinforced PTFE membrane, measurement and self adaptive automatic regulation by airflow variation resolution 0.1 mg/ml, automatic temperature compensation
Gas input:	max. air pressure 0.2 MPa,
Airflow:	airflow meter 0-5l/min. resolution 0.1l/min., linearity +/- 3% reproducibility +/- 0.5%, electronic and mechanic control through proprietary proportional needle valve
Sample collection:	4 cannulae with LAMBDA DOUBLE LOCK connectors
Remote control and data processing:	FNet or SIAM software for fermenters

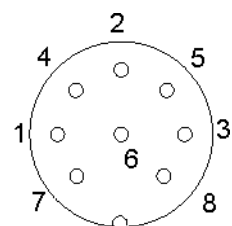
Inputs and Outputs:

Pump connection:
(8-pin connector)

Pin No.:

Wire colour cable:

1- Input remote control + 0/10 V	(yellow)
2- Step frequency of pump motor (0 and 12V)	(grey)
3- Power supply 0 V	(green)
4- Power supply + 12 V	(brown)
5- Pump remote control ON/OFF (0 to +12 V)	(white)
6- RS485 GND	(pink)
7- RS485 B (-)	(red)
8- RS485 A (+)	(blue)



PC-connector: 9-pin

Electrodes: pH and temperature, cable with VarioPin connector

Oxygen: BNC connector

Additional probe: BNC connector (antifoam, pCO₂, etc.) Input: 0-10V

Alarm-Output: Audio connector 12 V/0.1 A

8. ACCESSORIES (OPTIONAL)

- Connector for controlling sample collector OMNICOLL and a pump (Art. No.: 6911)
- PRECIFLOW pump (Art. No. 4801), MULTIFLOW pump (Art. No.: 4901), HiFLOW pump (Art. No.: 5001), MAXIFLOW peristaltic pump (Art. No.: 6001)
- MASSFLOW gas flow controller 0-5l/h or 0-500ml/h (Art. No.: 800701 or 800702)
- Pump connection cable (Art. No.: 6910)
- Peltier element for outflow cooling (air output condenser)
- Oxygen electrode
- Other accessories and spare parts are found on: www.lambda-instruments.com

9. 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:.....

LAMBDA Laboratory Instruments

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10. APPENDIX

10.1 Over-pressure safety valve

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, for example 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 attain 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



For the above-mentioned reasons the use of the delivered pressure limiting security valve is imperative!

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 not normal. At this moment, the input air pressure should be decreased and the output filter exchanged or a second new one installed.

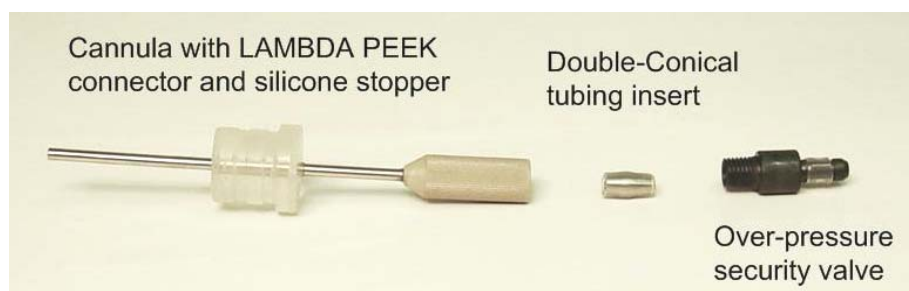


We have intentionally preferred this solution to the commonly used burst membranes. Burst membranes, besides of being very expensive, open immediately the vessel and medium is contaminated and consequently the culture cannot be saved. With our approach the run can be usually saved.



Maximum input pressure must not exceed 0.2 MPa even during short time! If this happens the air tubing inside the MINIFOR fermenter will burst and must be replaced!

Over-pressure 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 critical pressure is attained the tubing extends and air escapes.



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/temperature probe

How to use the LAMBDA pH/temperature sterilizable probe with nano-suspension:



Fig. 20: pH/temperature probe with nanosuspension: Type LA801 (Art. No: 800053)

The LAMBDA combined pH/temperature sterilizable probe LA801 (Art. No. 800053) is a glass electrode combined with Ag/AgCl reference which uses a new type of electrolyte. The KCl is permanently immobilized in a **nanosuspension** of highly pure and inert material. This new system brings solution to problems encountered with gel filled systems. There is no need to refill the probe with fresh electrolyte. Any formation of silver compounds on the frit of the reference electrode is suppressed.

Temperature is measured by a highly precise, miniature Pt100 element placed in the pH measuring part of glass electrode. This brings fast and precise measurement of the temperature, what is of major importance for the final precision of temperature control. The probe is equipped by high quality **Variopin** connector which can be sterilized without any cap.

Precautions to take for correct sterilization and using of pH probes

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 short-cut the pH signal or even build-up electrochemical potentials. The measured pH signal might then even lead to reading of pH 0 or 14, or stay constant around pH 7 due to a short-cut of the glass electrode signal. The tiny layer of dirt between the contacts can even change in time and result in erroneous pH readings.

In such a case it is usually necessary to change the whole cable with the connector. It is therefore necessary to observe an **absolutely clean work** with the pH probe. LAMBDA and no other producer can change laws of chemistry and physics so the probes must be treated with utmost care.

No cable survives very harsh conditions of sterilization. Therefore, each pH probe (and all other probes) must be disconnected from its cable during the sterilization in an autoclave.

LAMBDA has selected the best known pH probe connector. It is the Swiss made connector Variopin. Variopin is expensive, but because of its quality it became an industrial standard in quality instruments.

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 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 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 in the autoclave is relieved too fast, the autoclave water may boil over and contaminate the connector of the pH probe. Since the autoclave water is frequently dirty, it can contain salts, medium and other contaminants which can contaminate the pH probe. Thus, **take always great attention to clean pH probe connector before inserting the probe into the cable connector.**

If you observe an unusual pH reading please consider the above described information. Sometimes it can be possible to save the contaminated pH cable connector. This is, however, a risky task. Please, contact us if you need advice.

Measurement range:

pH: 0 to 14
Temperature: 0 to 100 °C (sterilization up to 130°C)

Measurement:

Remove the protection band from the tip of the electrode. If necessary shake gently the probe 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.

Storage of the probe:

To extend the life time of the probe keep the lower part in diluted buffer of 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 ball with grease, organic solvents, strong acids and bases.

Sometimes small cracks of the reference electrolyte or air bubbles may appear. This is normal.

Warranty:

The probe is covered by the warranty during 9 months after delivery for all defects due to production errors. The probe has to be used according to the above described instructions.

Cleaning of the probe:

Sometimes the surface of the glass electrode must be cleaned. Any of the following procedures may help to “save” the contaminated electrode.



Using of these treatments, however, leads to the loss of our warranty.

Contamination by lipids:

Use detergents, solvents (ethanol, acetone, diethyl ether (short time only)). Then wash with distilled water and leave the probe in KCl or tap 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 added by 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.

If the diaphragm is blocked it can be cleaned by gentle mechanic removal of the thin blocking layer by small fine file or fine sand paper. The glass electrode must not be touched. Equilibrate in 1M KCl.

10.3 pO₂ probe: Sterilizable sensor for dissolved oxygen (DO)



LAMBDA has developed an oxygen probe using complete non-metal body made of a new very resistant material PEEK, which has similar chemical resistance to PTFE, but is mechanically much more stable. The electrode is of Clark type with large Pt cathode and Ag/Cl reference anode. The membrane is made from glass reinforced thin layer of PTFE. The PTFE is better than silicone membranes, because much less deposits form on Teflon surface than on any other material.

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

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

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

Other factors, such as atmospheric pressure and salinity are of small importance in fermentation and are usually neglected.

Polarization: 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. LAMBDA oxygen probe has short polarization time. It is advisable to keep the probe connected to MINIFOR in stand by condition.

Measurement: Place the tip of the probe about 1 cm from the edge of the closest stirring disk. This will assure 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. (Because of the fast response of the probe this can be seen as signal variation especially at low DO concentrations).



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

Membrane replacement: When the electrode shows 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 small amount of mild detergent. Finally wash the membrane with distilled water. If this will not help, it may be necessary to replace the membrane (see also section 10.4).

If the reaction is fast but the signal is unstable, it may be due to the perforation of membrane. This requires also its replacement.

Unscrew the membrane module; wash the anode and cathode with distilled water. Dry the cathode (platinum circle diameter 1 mm on the tip of glass body) with soft paper. Add 10 drops of electrolyte (art. no. 800097 DO probe solution) into the membrane body. Shake the electrolyte down in the tip direction and screw the new membrane module on the slightly inclined body keeping the air escape groove upwards. Screwing should be slow toward the end so that the air has time to escape.

Technical properties of the DO probe:

- pO₂ 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
- polarisation time less than 2 hours

10.4 DO probe: Instructions for electrolyte and membrane module exchange

When the electrode shows 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 small amount of mild detergent. Finally wash the membrane with distilled water. If this will not help, it may be necessary to replace the membrane.

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 membrane. This requires also its replacement.



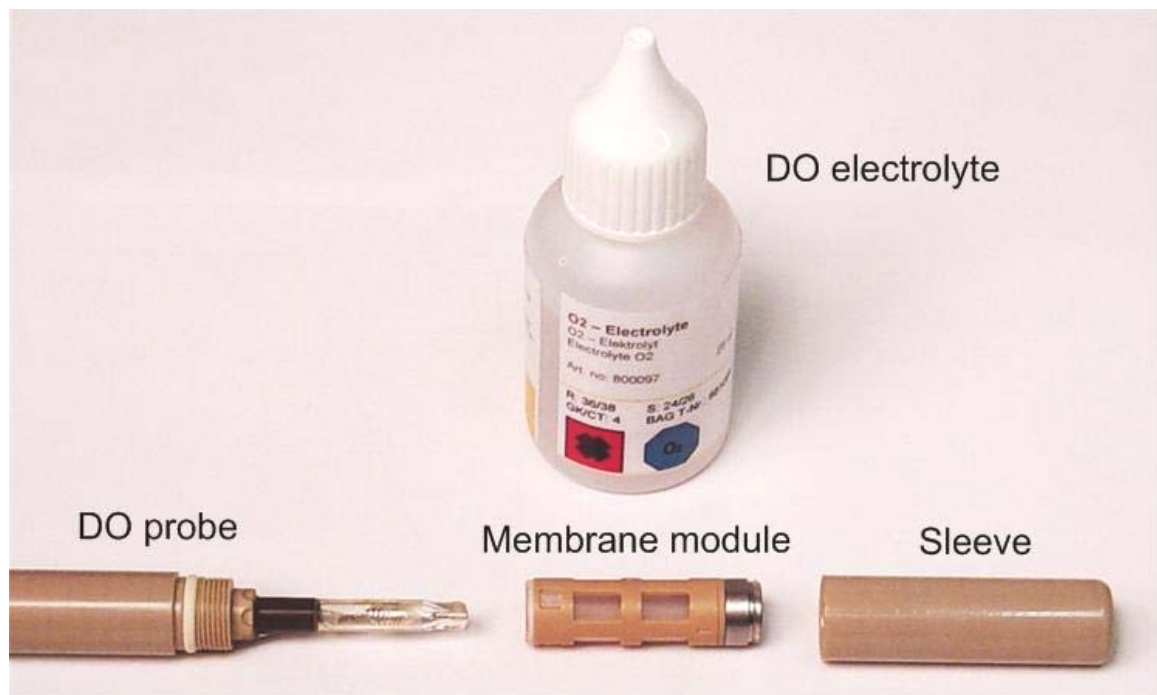
The pO₂ electrolyte is an alkaline solution (pH=13). Contact with skin, especially mucous tissues or eyes, should be avoided. Because contact with the electrolyte is very likely during exchange of electrolyte or membrane module, the use of protective gloves is recommended. In case of contact, the place should be well washed with lot 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.



This DO electrode can be used with the standard INGOLD-METTLER membrane module: Type T 96



DO probe components: DO probe, membrane module, sleeve and DO electrolyte.

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 on 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 repolarized and recalibrated (see the corresponding section of the LAMBDA MINIFOR manual).



The DO probe membrane is very thin and therefore very sensitive to mechanical contact. Handle it very carefully.

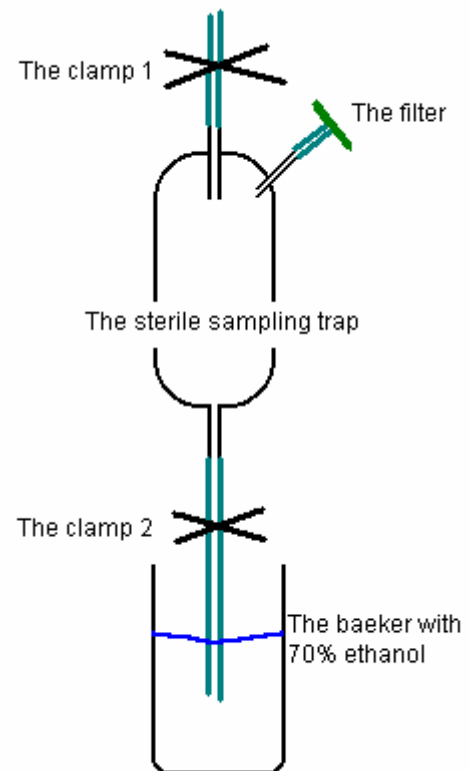
10.5 Sterile sampling device

To decrease the risk of contamination during sample withdrawal LAMBDA supplies an easy to use device. It consists of glass trap with three inlet/outlet tubes. Samples are taken from the vessel using one port of the four port sampling assembly (the one with the longest needle). The inlet tubing is fixed to the vertical inlet tube.

A sterilizable air-filter is connected to the side tube by short silicone tubing and silicone tubing 10 – 20 cm long is connected to the output tube.

All is sterilized in an autoclave together with the vessel. After sterilization the sampling trap is fixed in the supplied holder (for ex. on the vessel support rod see picture).

All tubings are clamped immediately after sterilization. The output tubing is conveniently dipped into a beaker with 70% solution of ethanol in water (this assures sterility of the output tubing).



Sampling procedure:

- remove the clamp 1 from the inlet tube (leading to the vessel)
- remove the clamp from the tubing leading to filter
- by means of syringe (50 ml) connected to the sterile filter pull the air from the trap. This will transfer small amount liquid from the vessel into the sampling trap. (This has to be done smoothly and the sample should flow in thin but continuous stream (if possible without drop forming) into lower part of the trap. Bursting of drops may transport small parts of sample on to the inner inlet tube). **Never fill the trap so much that the liquid touches the inner part of the input tube!** If you need more sample volume, take it twice.
- clamp the input tubing 1, remove the output tube from ethanol. Shake the drop of this solution away, place the output tubing into the tube, in which you would like to get your sample
- remove the output clamp 2 and with syringe push air trough the sterile filter into the trap. This will transfer the sample into the sample tube.
- clamp all tubings again and place the lowest part of the output tubing into ethanol solution.



It is possible to push the medium present in the line back to vessel. We normally do not recommend this, because in the case of contamination in the trap you may contaminate your vessel as well. We recommend proceeding with a line washing before taking the next sample. The procedure is the same as that for taking the sample, but the solution (washings) is not used as sample.

10.6 Oxygen saturation in water

Calculation of the saturation concentration of oxygen in water:

- agitate and aerate medium in the vessel
- read the temperature (after its stabilisation)
- find out your altitude above sea level
- get the relative air pressure (weather forecast) (if you do not know use the value 1013)

Use the following equation:

$$\text{Calibration value } C = S \times K \times L$$

where

- S is the standard oxygen saturation value at your temperature,
- K is the altitude correction factor from the table 2
- L is the ratio = relative air pressure/1013

Example:

Your calibration temperature is 18°C
 Your lab is located at an altitude of 500 m
 Atmospheric pressure is 1022hPa

Therefore S = 9,45 mg/l, K = 0,943, L = 1,0089

Your calibration value is 8,99 or 9,0mg DO/l

Table 1: Oxygen saturation in water at various temperatures in mg DO/l at standard air pressure 1013 hPa (value S).

°C	mg O ₂ /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 2: 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		

10.7 Scale module for continuous cultures (chemostat)

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 (art. no.: 800800) for LAMBDA MINIFOR laboratory fermenters and bioreactors. It allows keeping the amount of medium constant and independent of the extent of aeration, foaming and stirring.



This is a much more precise method for the control of medium volume than 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. Fig. 20 shows the weighing module (scale module) for continuous cultures:

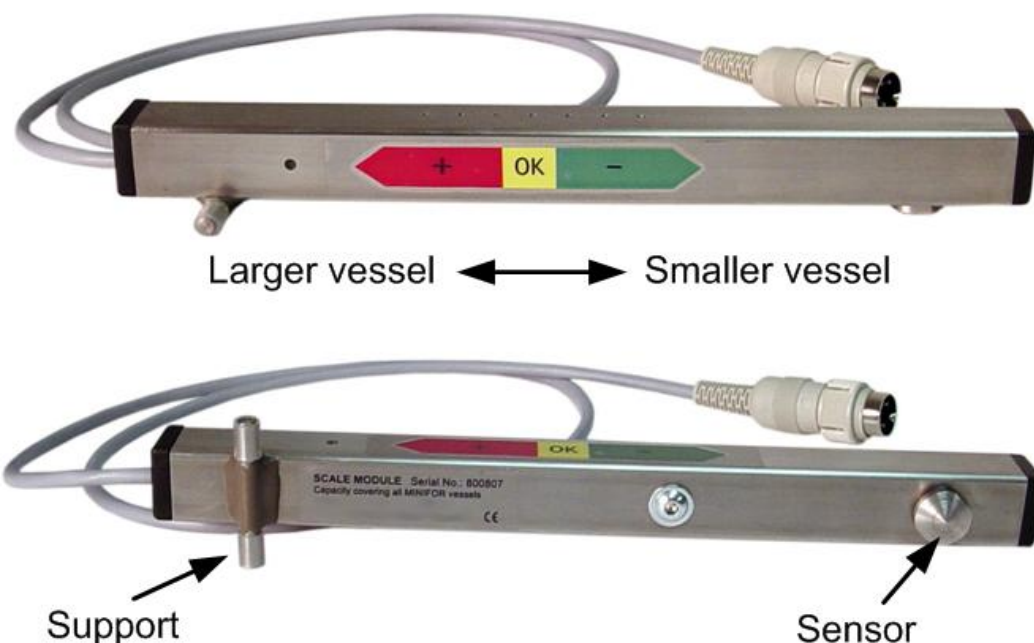


Fig. 20: Weighing module (scale module) used for continuous fermentation and cell cultures



All versions of MINIFOR fermenter-bioreactor are already wired and equipped with regulators permitting the connection of the LAMBDA scale module.



The scale module contains a sensitive weight sensor. Avoid any shocks of the sensor and handle it with great care.

Installation of the scale module

The scale module is placed under the front part of the MINIFOR fermentor base unit:

- The instrument weight is transmitted through the tip mounted under the front edge of the MINIFOR fermenter base unit. In this hardened steel tip the weight is concentrated.
- The miniature scale module with all necessary electronic circuits is located in a square tube assuring highest mechanical rigidity. There are several conical holes on the upper face 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 5 l vessel.
- When small, light vessels are used select holes close to the weight sensor (away from the connection cable, see Fig. 20). This will increase the sensitivity of the weight control. Use holes on the other side (close to the cable, see Fig. 20) for heavy large volume vessels. Through this selection of the holes the lever effect between the support and the sensor location (see Fig. 20) is used to increase or decrease the sensitivity of the scale module. Thereby, the measuring range can easily be adapted.



Connection of the scale module to the MINIFOR fermenter-bioreactor

The scale module is connected into the “X” socket on the left side of MINIFOR base unit. Both supply voltage and signal lines are combined in the same cable. This simplifies the set up, eliminates one cable and frees the “PUMP” socket at the rear of the MINIFOR for other uses.



Range selection

- Connect the grey cable connector to the socket “X” on the left side of MINIFOR casing.
- After this connection the scale module gets activated and the actual weight signal will appear on the display in the first row of parameter “X”.
- If the weight of the **filled** vessel exceeds the measurement range, a roof signal “^” will appear before the measured weight value. Put the weighing tip of the MINIFOR to the next hole towards the scale module connection cable. If the sign of overweight “^” still remains, move the weighing tip again to the next hole closer to the cable side. Repeat this procedure until the overweight sign disappears.
- If the vessel weight is too low, an underscore sign “_” will appear before the measured weight value. Move the weighing tip under the front of MINIFOR closer to the sensor of the scale module. If the sign of underweight “_” still remains, move the weighing tip again to the next hole on the left. Repeat this procedure until the underweight sign disappears.
- On the scale module the range adjustment is also indicated by the color of LED placed on the front side of the weighing module. The optimal position is obtained when the color remains yellow (wait until color becomes stable before the next adjustment step).
- When no over- or underweight range sign appears you can start the **calibration**. The advantage of the present measurement system is that it is not necessary to measure the

whole fermentor weight, but just select the range where the measurement or volume control is required. This results in a more precise measurement.

Setting of the sense of regulation

This function allows selecting the logics of the “X” pump function. It can be either switched on after the preset value is attained or alternatively it can be switched off.

When the signal LED of the fermentor front panel of MINIFOR is yellow (selected by button **R**), press button **C** (calibration). The message “UP” or “DOWN” is displayed before the “X” column. Press the up or down cursor so that the setting “DOWN” appears. Press the right cursor arrow to memorize the selection.

The weight can be controlled either by addition (“UP”-mode) or removal (“DOWN”-mode) of medium solution.

Calibration (with filled vessel)

With the MINIFOR fermenter in **calibration mode** move the cursor into the “X” column. Type for example 100 and press the right cursor button.



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.

- Observe the actual value of the measured weight. When the reading becomes stable wait until the message “**HOLD**” appears before the actual weight reading. Then press button **C**. *(During the “HOLD” time the weight variation is not memorized and therefore your finger force will not affect the calibration).*
- Then place a known weight or add a certain known volume of liquid to the vessel. Let us say 100 g or ml.
- Type 200 in the X column and wait until the reading becomes quite stable (about one minute). Wait until the message “**HOLD**” is displayed and press the calibration button **C**. The message “**OK**” will appear indicating that your calibration has been accepted by the MINIFOR fermenter. The scale is now calibrated in g or ml. You can make other weight calibrations according to your need (for example dozens of g or other weight units).



It is practical to have a piece of metal of suitable weight which is placed on the fermenter next to the vessel for calibration.

Weight setting

- Press the button **R** to exit the calibration mode.
- Place the cursor to the second row (set value) of the parameter “X” column.
- Select the desired weight to be kept constant, e.g. 100 or 200 or any other required value and confirm it by pressing the right cursor arrow.

Connection of the withdrawal pump

- Connect the corresponding pump to the “PUMP X” ^{*)} socket on the rear of fermenter-bioreactor MINIFOR. Press the **ON/OFF** and **REMOTE** buttons on the pump.
- When the culture or fermentation is started by pressing the button **R** 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.

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

10.8 LAMBDA ANTIFO anti-foam detector and controller

The LAMBDA MINIFOR laboratory bioreactor-fermenter can be equipped with a novel foam detector and control system (see Figure 1). The presence of foam in the reactor vessel is detected by measurement of electrical conductivity. The electrical conductivity can be measured readily and no expensive anti-foam probe is required. Instead of such an antifoam probe two needles of the quadruple port of the LAMBDA MINIFOR fermenter vessel are used. As a consequence, no additional port has to be used for anti-foam detection and control.

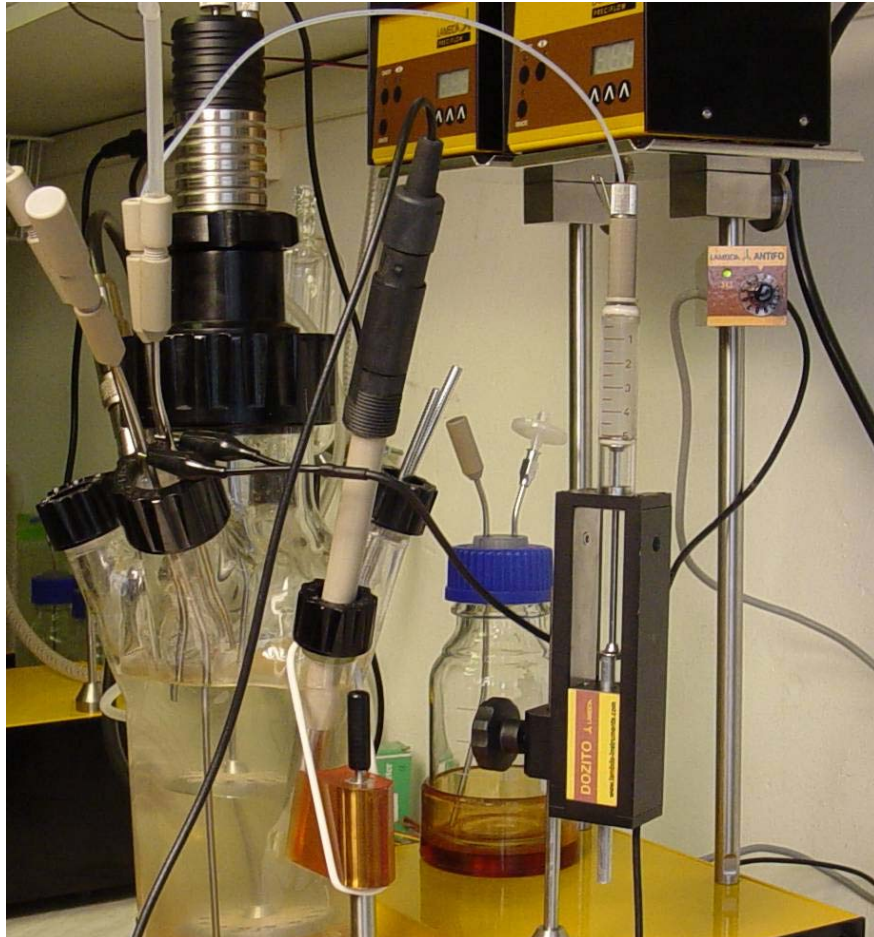


Figure 1: Antifoam detection and control system of the LAMBDA MINIFOR fermenter/bioreactor.

Setting up the LAMBDA ANTIFO foam detector and controller

Place the main body of the ANTIFO anti-foam controller on one of the support rods on the rear of MINIFOR laboratory fermenter/bioreactor, e.g. under a pump carrying support. In this way, it will be well accessible and occupy minimal space (see Figure 2).

Connect the grey eight-pole connector to any unused pump socket at the rear of the MINIFOR fermenter base unit casing. In case all sockets are already used you can use the optional quadruple plug box (Art. No.: 800202) for the connection (see section 10.10).



Figure 2: Placement of the antifoam controller.

The harvest needle of the quadruple port, which reaches to the bottom of the vessel, is used as the medium contact. Therefore, connect one crocodile clip to this needle. The second (foam) crocodile clip can be connected to any of the three shorter needles of the quadruple port. Adjust the tip height of the “foam”-needle to the position above medium level where the foam should be controlled. Put the second crocodile contact to the selected short needle (see Figure 3).



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

Each culture medium, depending on its salt content, produces certain background conductivity. This conductivity will occur even without the formation of foam and it should be taken into account by setting a medium specific conductivity threshold. Before doing this conductivity threshold setting, a thin film of medium should cover the inner walls of the reactor vessel in a way which will correspond to normal culture conditions:

1) Start agitation at the maximal used speed of the planned process and wait about one minute until the walls of the vessel are wetted by the culture medium.

2) Adjust the sensitivity threshold for the given medium by rotating the knob until the light of the SET LED diode just switches from yellow to green (see Figure 4).

When the foam level reaches the non-immersed tip of the selected foam detection needle, the electric conductivity will increase and the ANTIFO anti-foam controller output will be activated (the LED will change from green to yellow) to start the addition of the antifoam agent by the miniature syringe pump LAMBDA DOZITO (see section 10.9). This DOZITO syringe pump is connected to the ANTIFO anti-foam controller by connecting the cable plug into the corresponding socket of the ANTIFO controller casing.

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 substance occurs only when the foam did not disappear during the elapsed waiting interval time. Additionally, the anti foam dose can also be controlled by the volume step setting on the LAMBDA DOZITO miniature syringe pump (see section 10.9).

Technical parameters

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



Figure 3: Connection of both electrodes.



Figure 4: Control panel of the ANTIFO-controller.

10.9 LAMBDA DOZITO miniature anti-foam syringe pump

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 the compact antifoam detector/controller and probably the world's smallest syringe pump system (see figure on the right).



Preparation of the anti-foam line

A special frequently 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 screwed on the syringe side.

In the following, the setting up of the syringe pump is described step by step (see also the pictures below):

- 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 small internal diameter if you use viscous liquids*). The parts needed for the assembly are shown in (a).
- Push the pusher of syringe completely to the bottom of the syringe to mechanically fix the metal outlet tip (b).
- Place the small silicone washer around the metal tip (b).
- Put the thin PEEK washer on the connection body (c) and screw it to the metal tip of the syringe. This should assure tight connection of the assembly. (d)
- Push the tubing through the double conical silicone part so that about one to two mm of tubing reaches out. (e) (*Use water or light silicone oil to make the sliding easier.*)
- Put the metal fixing wire on the threaded connector and screw all together. This makes a tight connection of PTFE tubing with syringe. (f) and (g)
- Place another threaded tube on Teflon tubing (with thread facing the end of this tubing).
- Push another double conical silicon joint about 15 cm from the tubing end. This length should long enough to allow that the tubing tip will reach out of the needle.
- Fill the syringe with the antifoam solution. (Viscous liquids are preferably filled by removing the pusher, pouring the liquid into the syringe and reinserting the pusher in usual way with elimination of air in vertical position). It is preferable to fill the whole length of tubing with antifoam liquid. (i)
- Attach the syringe assembly on one needle by the wire hook (this conveniently eliminates the folding of the tubing) (j), then push the tubing through selected needle and screw it in the desired position. (k)



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



a)



b)



c)



d)



e)



f)



g)



h)



i)



j)



k)



l)

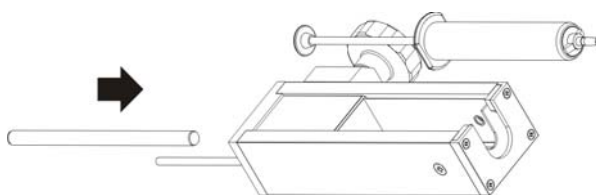
Sterilization procedure

For sterilization the syringe is placed into the sterilization support (see picture h). The syringe plunger is fixed by a screw to prevent its movement during sterilization. The syringe is hooked on any needle port and is autoclaved together with the fermenter vessel (see picture j and l).

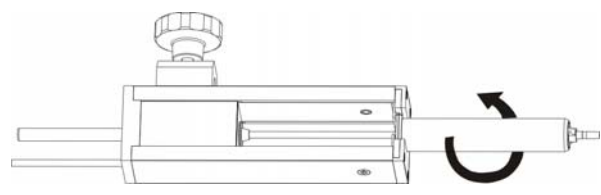
After sterilization the syringe is removed from the sterilization support and placed into the head of the DOZITO syringe pump (see next paragraph).

Inserting the syringe into the LAMBDA DOZITO miniature syringe pump

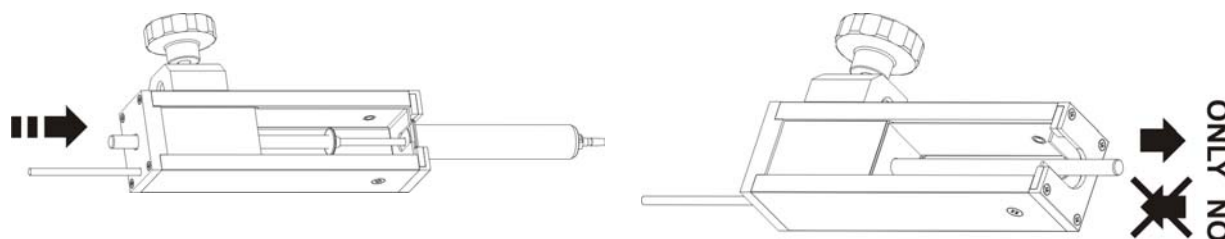
The syringe can now be inserted into the miniature LAMBDA DOZITO syringe pump as shown in the following figure:



A) Introduce the syringe into the syringe pump fixing slot (by the narrow syringe side) and insert the pusher rod into the syringe pump in the direction indicated by the arrow.



B) The syringe is secured in place by rotating the syringe body by 90 degrees (a quarter turn) as indicated by the arrow.



C) The plunger of the anti-foam syringe is pushed until the antifoam liquid just comes out the PTFE tubing tip. During anti-foam addition the pusher rod moves the syringe plunger in the direction indicated by the arrow. It is also possible to manually add antifoam by moving the pusher rod in the same direction (arrow).

D) After the syringe plunger has reached the end or after the process is terminated, the syringe is removed from the syringe pump by rotating the syringe by 90° in the opposite direction as in step B) and removing the syringe from the syringe pump. **The pusher rod is pushed out of the pump in the indicated direction only. Moving the rod in opposite direction will damage the syringe pump.**

First, the syringe is located by the narrow syringe side into the syringe pump fixing slot. Introduce the metal pusher rod into the syringe pump in the direction indicated by the arrow until it slightly touches the syringe pusher (picture A).

The syringe is secured in place by rotating the syringe body by 90° (picture B).

The plunger of the anti-foam syringe is pushed by the pusher rod until the antifoam liquid enters the PTFE tubing tip (filling the anti-foam line). During the anti-foam addition the pusher rod moves the syringe plunger in the direction indicated by the arrow (picture C). By pushing the pusher rod by hand, it is also possible to manually deliver antifoam agent into the reactor vessel.

Fix the DOZITO syringe pump on the rod of the magnetic stand and secure it in position by the screw knob. The magnetic holder can be put at any convenient place on the upper cover plate of the MINIFOR fermenter/bioreactor base unit casing (see picture on the right).

After the process, the LAMBDA DOZITO syringe pump is removed from its magnetic stand. If necessary, the metal pusher rod is removed from the DOZITO syringe pump body (picture D). **Never push the moving rod in the opposite direction as this will destroy the mechanics!** To remove the pusher, just push it further in the same direction (arrow). Then it can be introduced again into the syringe pump from the outside (picture A).



Always remove the pusher rod in the sense of the syringe pusher movement. Never push this rod back into the body of DOZITO syringe pump, because it will be damaged.

Setting of the delivered anti-foam agent volume (volume step)

The volume of added anti-foam liquid can be varied from a dozen of micro-liters to about 0.3 ml. This volume setting is done by screwing in (clockwise rotation = decrease of the delivered anti-foam volume) or out (counter-clockwise rotation = increase of the delivered antifoam volume) the small screw on the head of the DOZITO miniature syringe pump (see picture on the right).

The corresponding key is delivered with the instrument. The screwing must be done with sensitivity; otherwise the end sensor might be damaged. This volume adjustment screw is protected against complete screwing out.



Function indicator

On the side of the LAMBDA DOZITO miniature syringe pump there is a LED which indicates the operation state of the syringe pump. After receiving the control signal from the ANTIFO anti-foam controller, this LED lights yellow and indicates that the pusher moves and delivers the pre-selected volume of antifoam agent.

When the preset dose has been delivered the LED will switch to red. Then the communication with ANTIFO is interrupted during approximately 20 seconds and the indicator LED is turned off.



Technical parameters

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

10.10 Quadruple plug box

The quadruple plug box (Art. No.: 800202) allows the connection of up to four additional LAMBDA instruments (e.g. pumps, mass flow gas flow controller, weighing module, antifoam controller,...) to the LAMBDA MINIFOR laboratory fermentor/bioreactor. This plug box provides 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.



Fig. 21: Quadruple plug box for connection of additional instruments to the LAMBDA MINIFOR laboratory fermentor-bioreactor.

10.11 Setup of the agitation/mixing system

In the following, the setup of the agitation/mixing system is presented step by step:

Make all agitation system components ready for the mounting.

Stirring discs and self-cleaning microsparger have been placed on the stirring axis beforehand.



Introduce the mobile cock into the head piece.



Screw the air input tubing holder into the mobile cock opening.

The tubing should cover the double-conical stainless steel tubing inserts almost completely to guarantee perfect tubing sealing.

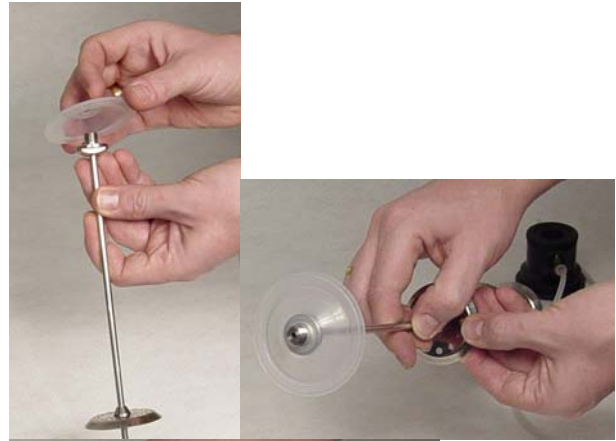
Pay attention to insert it at an angle of about 60°. The air input tubing holder should be placed parallelly to the upper part of the head piece



Tighten the air input tubing holder with reasonable force.



Put the large silicone sterility membrane on the upper part of the stirring axis as shown on the picture (so that the conical membrane will nicely fit in the conical shape of the mixing head).

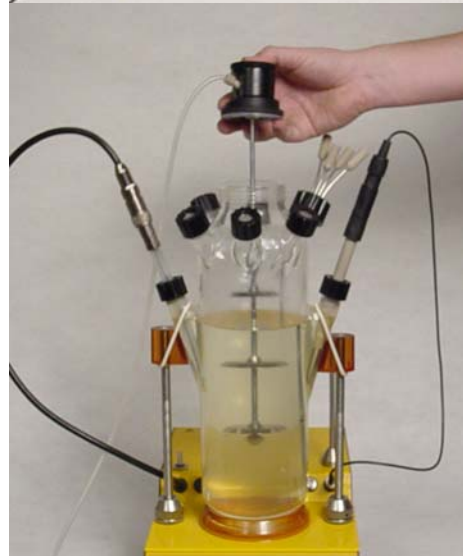


Screw the stirring axis manually or with a key to the mobile cock in the fermenter head.

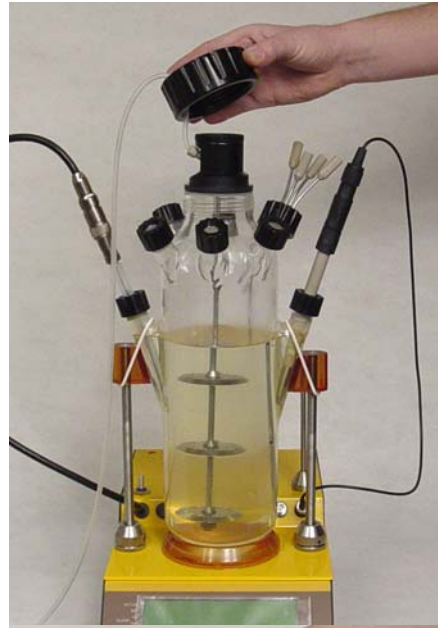


Put the stirring axis assembly into the fermenter vessel.

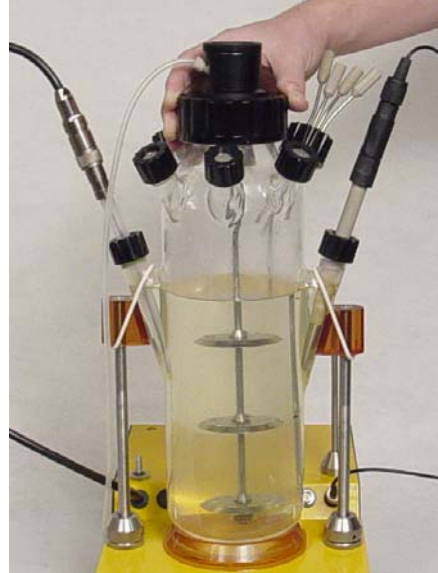
Take care to place the sterility membrane in central position on the central vessel opening (large threaded neck).



Put the large threaded cap on the fermenter head and allow the air input tubing to pass through the cap.



Tighten the cap well in position.



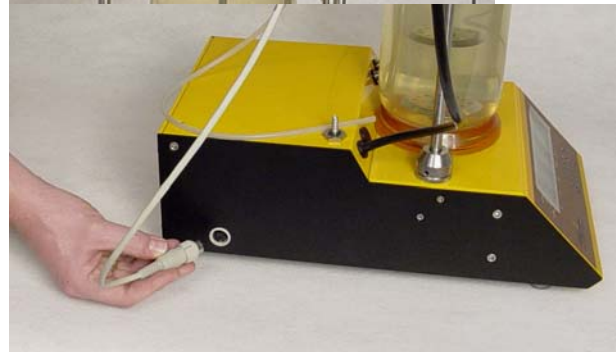
Place the motor unit onto the fermenter head. The magnetic coupling will engage spontaneously and fix the stirring axis in place.



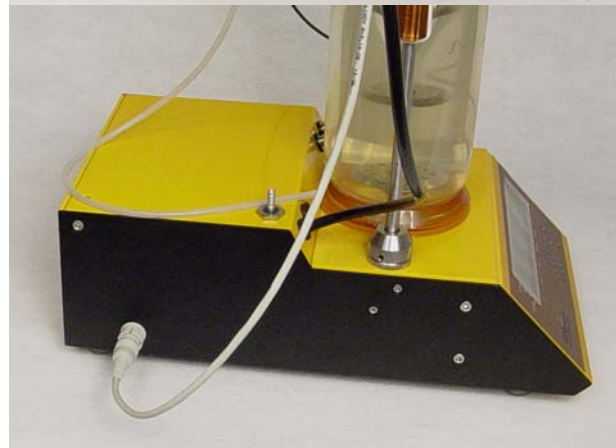
Fix the motor unit in position by the corresponding nut.



Connect the motor unit into the socket on the left side of basic unit.



Secure the plug in position by rotation of fixing ring.



10.12 Electronic 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.

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 water from the outgas stream than is obtained in water cooled condensers. Such an electronic condensing system also allows a more efficient and constant cooling **without requiring any cooling water!**



Electronic out-gas condenser assembly

Peltier cooling
element



Out-gas
condensing
finger



Out-gas condenser cylinder



Electronic out-gas condenser assembly: Peltier cooling element, out-gas condensing finger and out-gas condenser cylinder with tubing connection.

The out-gas condensing cylinder is inserted on the outgas condensing finger and screwed to the condenser body. Visually inspect the O-ring for mechanical defects and replace if necessary.

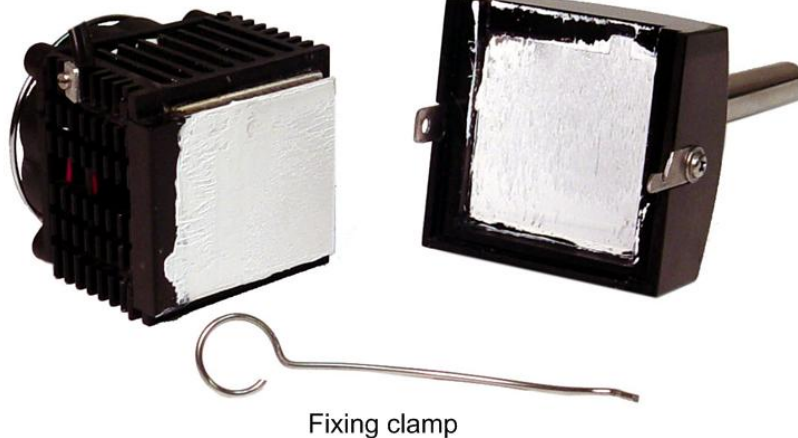
Put a small open screw cap, stainless steel washer and an open silicone stopper onto the stainless steel connection tube of the out-gas condenser cylinder.

The out-gas condenser assembly is then placed in one of the small side necks and screwed to the vessel. The tubing leading to the out-gas PTFE filter is fixed to the hose barb connector of the out-gas condenser cylinder.

Before sterilization the Peltier cooling element must be removed from the out-gas condensing finger by releasing the fixing clamp (see picture below). If necessary, wipe the heat conducting paste from the last run by a solvent, e.g. acetone.

Peltier cooling element

Out-gas condensing finger



Electronic out-gas condenser connection: Peltier cooling element, out-gas condensing finger and fixing clamp.



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

After sterilization, apply a small amount of heat-conductive paste (art. no. 800084-p) onto the squared out-gas condensing contact surface to guarantee a good heat transfer from the Peltier cooling element to the out-gas condensing finger. The amount of heat-conductive paste should be as small as possible to just cover the whole square surface.



The heat-conductive paste can be supplied by LAMBDA (art. no. 800084-p) or obtained in electronic shops. It is used also for the cooling of electronic amplifiers, processors and the like.

The **Peltier cooling element** is then firmly pressed to the metal plate of the outgas condensing finger and is kept in position by the fixing clamp. The tip of this fixing clamp is inserted through the cooling ribs into the hole in the fixing metal strip and clamped in by the hook on the opposite side.

The plug of the Peltier cooling system is 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).



A wrong connection is not possible thanks to mechanical pin coding of all connectors.

After switching on the Peltier cooling condenser an indication LED will light on, indicating that cooling goes on. When the temperature reaches approx. 5 °C, the LED goes off. The temperature remains regulated and there is no danger of complete freezing of water in the out-gas cylinder, which could block the out-gas flow.

10.13 LAMBDA Liquid O-ring

Standard O-rings have several disagreeable properties.

- They flatten during sterilization and thus the connection can become untight, which may result in contamination of the culture.
- If any dirt or filaments are closed under the o-ring then microbes have a large tunnel to get into the vessel.
- Large o-rings require large compression forces to be perfectly tight. The screwing of connection surfaces must be done perfectly regularly in order to eliminate higher compression on one side and lower compression on the other one. The resulting mechanical forces may put a non negligible 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 only partially hardening silicone resin filled with purest quartz powder of extremely fine (fume like) consistence.

The use of this liquid o-ring is very easy. One part of solution A (based on weight or volume) is mixed with one part of solution B. For example 2 g of substance A and 2 g of substance B are sufficient. Then, AEROSIL powder is added in such an amount until the mixture is no more flowing (the consistence is similar to a tooth paste).



This mixture is then applied on the whole circumference of the lower vessel part rim like a "spaghetti", e.g. with a syringe.



Place the upper vessel part onto the lower vessel part and fix them together with the segmented vessel closing belt. The paste fills any cavity between the joining surfaces and is completely gas tight and also does not generate any stress to the glass.



Now the usual work can go on. 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.

Moreover, this liquid o-ring can be reused many times. When the vessel must be opened for cleaning just push the upper part to the side while keeping the lower part in the vessel support. The cover will slide away. 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.