LAMBDA
LABORATORY INSTRUMENTS

LAMBDA MINIFOR

Laboratory Fermenter

OPERATION MANUAL

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LAMBDA Minifor

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 optimisation 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 more gentle 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As far as possible expensive pieces of equipment have been replaced by new high performance plastics.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 visualization and recording pumped volume

TABLE OF CONTENT

1. PRESETTING FERMENTER

1.1 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, pO_2, 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 pO_2 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.

1.2 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

1.3 Setting the temperature

Move the cursor by using arrows on the field for the temperature setting (SET ℃, 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.

1.4 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.

1.5 Regulation of pO_2

The concentration of dissolved oxygen (pO_2) is regulated by continuous (step-less) 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.

1.6 Calibration of probes

Calibration of pH-probe

See Probe calibration (Chapter 1.2)

Calibration of pO_2-probe

The calibration is similar to calibration of the pH probe.
For the 0%-level the gauging solution is a 5% aqueous solution of Na_2SO_3 (first gauging solution).
In place of this it is possible 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. It 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 l of water and at the corresponding temperature can be taken from the enclosed table (see annexes).

Calibration of X-channel

See Probe calibration (Chapter 1.2)

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 to set a desired weight, which is kept constant. The X pump is activated when the preset weight is exceeded.

Note:

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

1.7 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.

1.8 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 visualisation of the different parameters.

Since most PC have serial output with the interface RS232 a converter RS232/485 is necessary. The RS485 allows connection of dozens of different instruments by the same line, where as with RS232 only one instrument can be controlled. The suitable converter is a part of the fermentation software FNet or SIAM.

2. PREPARATION AND STERILISATION OF VESSEL

2.1 Preparation of vessel

2.1.1 Mounting of vibration mixer

The disassembled head is shown in Fig. 2.

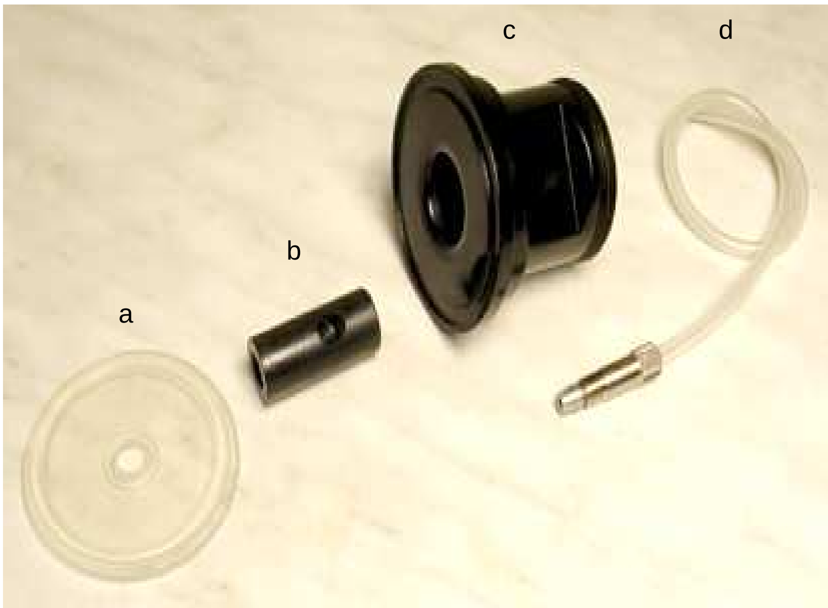


Fig. 2 Disassembled head of vibration mixer

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

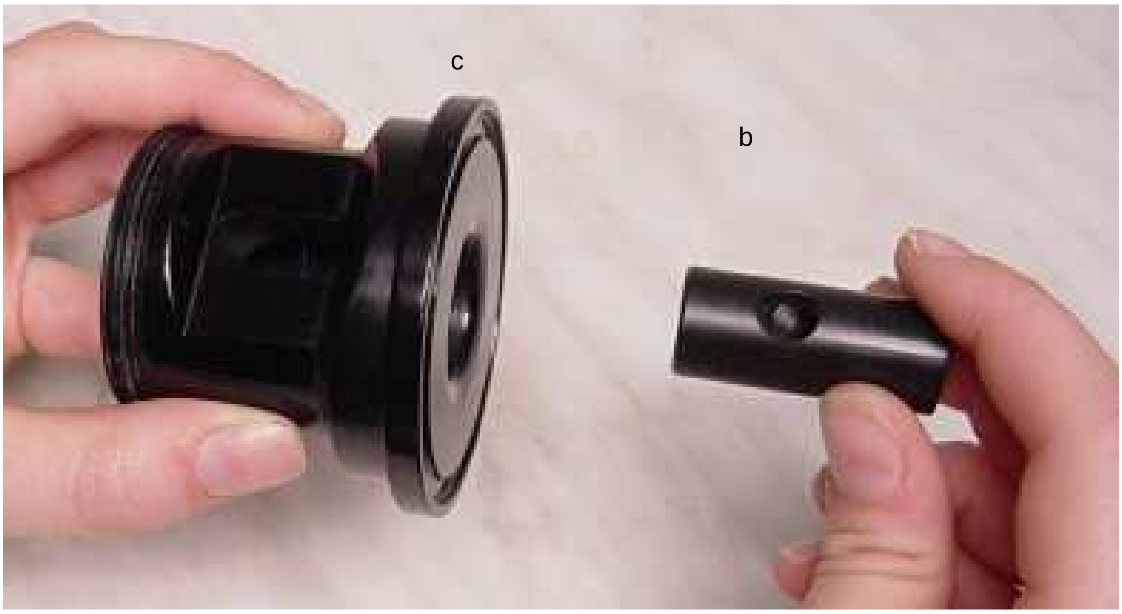


Fig. 3 Completation of the cock head

2.1.2 Air inflow

A silicone tube of 5mm diameter and 1mm wall thickness is used for the air inflow. The tube is put through the tubular jacket 5f. A doubly 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.

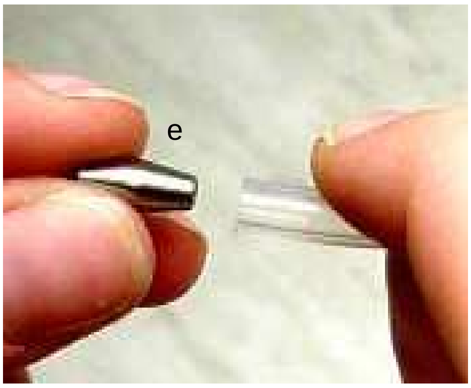


Fig. 4 A doubly conical insert is placed into the tube end

This assembly is screwed under inclination of 60° deeply in to 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 Tube 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 tube 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).

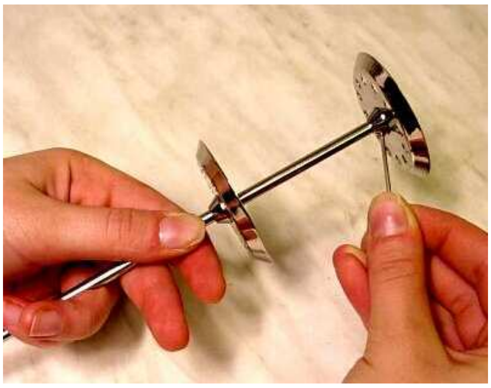


Fig. 8 The mixing disc is positioned on the axis

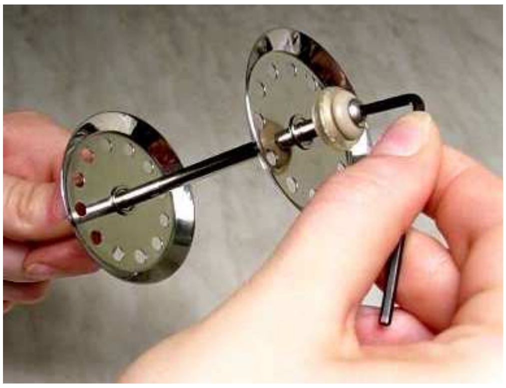


Fig. 9 The self-cleaning gas microsparger

The elastic material of our microsparger does not require any cleaning. If it should be blocked, the body of the sparger will inflate an the blocking deposit will split off.

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.

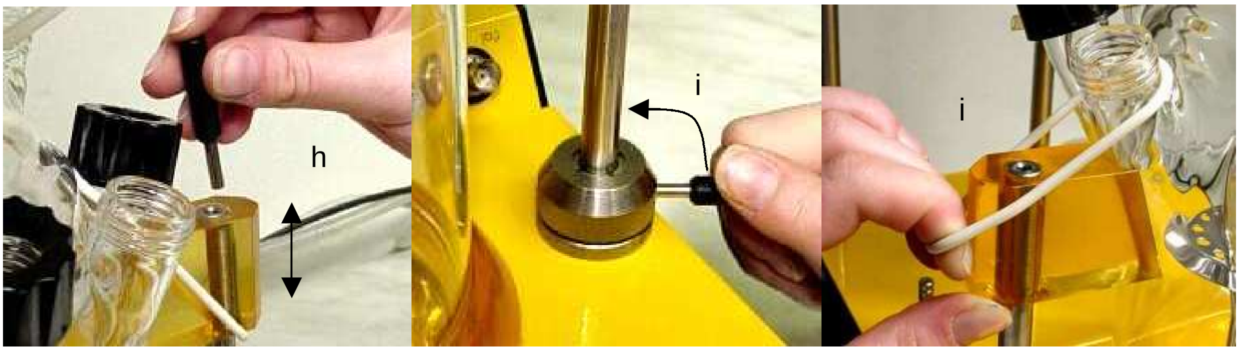


Fig. 11 Fixing the fermenter vessel onto the housing

2.1.3 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 The glass air outflow cooler

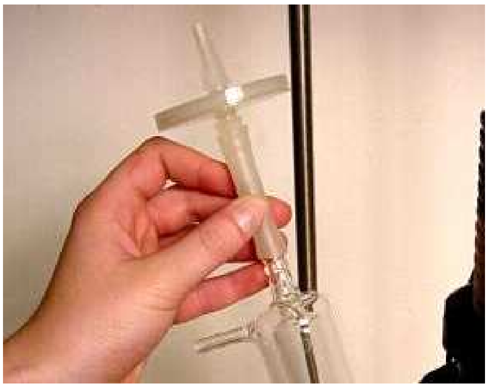


Fig. 13 The filter on the cooler

(Alternatively an electrothermic Peltier cooler can be used. It allows a much better condensation of water vapour because of low temperature attained (about 5°C). No cooling water is required.)

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.)

2.2 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 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.

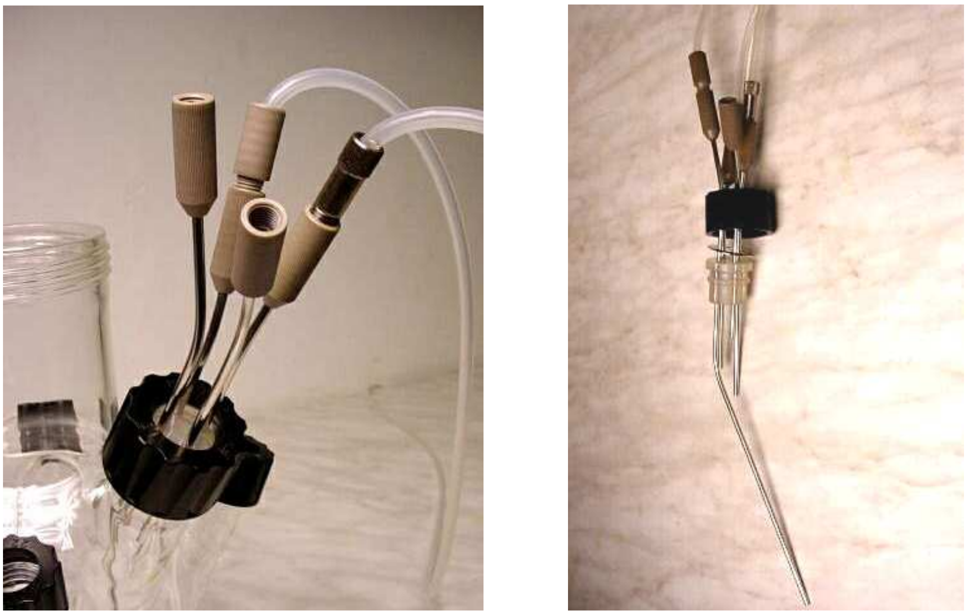


Fig. 14 Ports for additions and sample collection

(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.)

**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℃). Therefor e, 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.*

2.3 Probes

The pH-, pO_2- 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 pO_2 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 pO_2 probe (green plug)

ATTENTION:
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 contacts on both pH and pO_2 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 absolute clean. **Never put any cable into autoclave for sterilization!** This is similar to all sterilizable probes.

Remarks:

Before sterilisation, the vessel is filled with medium and all unused side necks are fitted with closed stopper, metal washer and a cap. The probe connectors have also to be protected with a cap, unless they are of the VarioPin type, which does not need protection.

2.4 Sterilisation

The fermenter vessel is sterilised as usual (e.g. 30 minutes at 120℃) and then cooled off. Generally, acid, base and the other solutions are sterilised 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.

ADVISES:

- 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 equalisation 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.
- Our DOUBLE LOCK Peek connectors can be exposed to temperatures up to 300℃ and can be flamed with precaution with alcohol burners for a short time. Silicone tubing can tolerate temperatures up to 250℃. If required metal connectors may be also supplied as option.
- Never put the magnetic bottle holders into autoclave for sterilization they will be destroyed!

3. START OF THE FERMENTATION

3.1 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 tighten 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 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 tubings are not shown here for simplicity.)

Fig. 18 Position of supply botles

- 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.

3.2 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.

4. 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.)*

- MINIFOR laboratory fermenter 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.

  - Bring the MINIFOR to calibration state.
  - Write Adr 99.
  - Press the right arrow
  - Press C button
  - Switch off MINIFOR
  - Switch Minifor ON again.

5. 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.

6. TECHNICAL CHARACTERISTICS

Compact microprocessor controlled laboratory fermenter MINIFOR

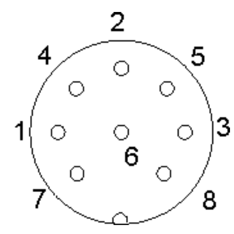
Display:	LCD 4x 40 letters
Power supply:	power line ~100-245 V /50-60 Hz, 400 W, CE conform
Operating temperature:	0 - 40 ℃
Relative humidity:	0 - 90 %
Security:	IEC 1010/1
Weight:	7.5 kg
Dimension:	22 x 38 x 40 cm (B x H x T) (mounted)
Utilisable Volume:	from 35 ml to 4 l
Vessel:	Pyrex 9 threaded necks
Mixing:	Vibromixer 0-20 Hz/30 W

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 nanosuspension, 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 largely protected glass reinforced PTFE membrane, measurement and self adaptive automatic regulation by airflow variation resolution 0.1 mg/ml, automatic temperature compensation
Gassinput:	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 programs 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	(rosa)
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, pCO_2, etc.) Input: 0-10V
Alarm-Output: Audio connector 12 V/0.1 A

7. ACCESSORIES (OPTIONAL)

- Connector for controlling sample collector OMNICOLL and its pump (Art. No.: 6911)
- PRECIFLOW pump (Art. No. 4801), MULTIFLOW pump (Art. No.: 4901), HiFLOW pump (Art. No.: 5001)
- Pump connection cable (Art. No.: 6910)
- Peltier element for outflow cooling
- Oxygen electrode
- Spare parts on request

8. WARRANTY

12 months on material defects or manufacturing defectives, if the device was used according to the operation manual. Condition for reparation is delivery to our repair service with a detailed description of the failure. Transport costs and risks are for the customer’s account.

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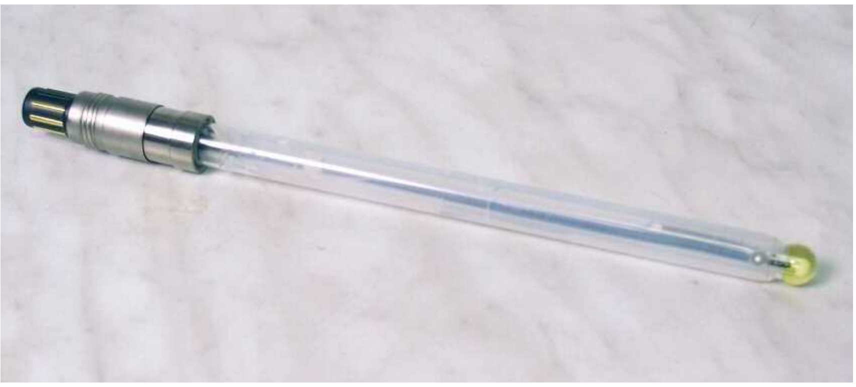
9. ANNEXES

9.1 pH Probe:

How to use Lambda pH/temperature sterilizable probe with nanosuspension.

TYPE LA801
Cat. No: 800053



The Lambda combined pH/temperature sterilizable probe LA801 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.

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.

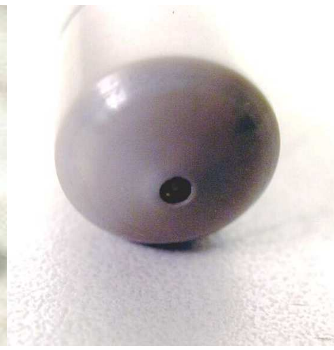
Contamination by proteins:
Leave the probe in the 0.1M HCl containing 10 mg pepsin/ml for several hours and wash with water.

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.

9.2 pO_2 Probe: Sterilizable sensor for dissolved oxygen

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 then 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.

Principe of function: 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.

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

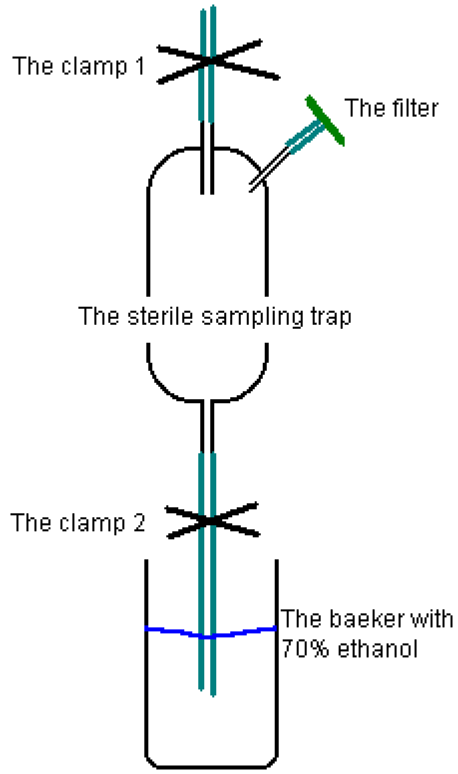
If the reaction is fast but the signal is unstable, it may be due to the perforation of membrane. This requires also its replacement.

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 (cat. 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:

- pO2 probe can be sterilized up to 130°C.
- short response time less than 2 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 hr

9.3 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. Sterilizable 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 take it twice).
- clamp the input tubing1, 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 like to this, because in the case of contamination in the trap you may contaminate your vessel as well. We recommend 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.

9.4 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 see level
- get the relative air pressure (weather forecast) (if you do not know use the value 1013)

Use the following equation:
Calibration value C = S X K x 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℃
Your lab has altitude 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).

℃	mg O2/l	℃	mg O_2/l	℃	mg O_2/l	℃	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 see level (value K).**

high (m)	K	high (m)	K	high (m)	K	high (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		