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User manual filtration probe for the installation
in a 25 mm side port

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Figure 1: Filtration probe for the installation in a 25 mm side port with membrane protective cage

1. Introduction

The filtration probe is intended for harvesting cell-free filtrate from bioreactors and fermenters under sterile conditions.

A tubular microfiltration membrane made of polypropylene, positioned inside the bioreactor, serves as a sterile barrier.

The filtration probe is sterilized together with the bioreactor.

The filtration probe is installed in a 25 mm sideport of the bioreactor.

Sterile cell-free filtrate is sampled continuously or discontinuously at the probe head that is accessible outside the bioreactor. For pumping the filtrate, the probe is connected to an external peristaltic pump. For manual sampling conventional single-use syringes can be used.

Because of the consumption of fermenter medium, the filtration probe is better suitable for big fermenters above 100 liters (in particular for microbial processes). The filtration probe is also suited for small fermenters, if the sampling intervals are long (e.g. in cell cultures).

2. Description of assembly

The filtration probe consists of a filtration unit (1) with membrane mounting (2) for the installation in a 25 mm side port, cap nut (3), shut-off cock (4), ferrule (6), o-ring (7), polypropylene membrane (8) and blind plug (9), see figure below.

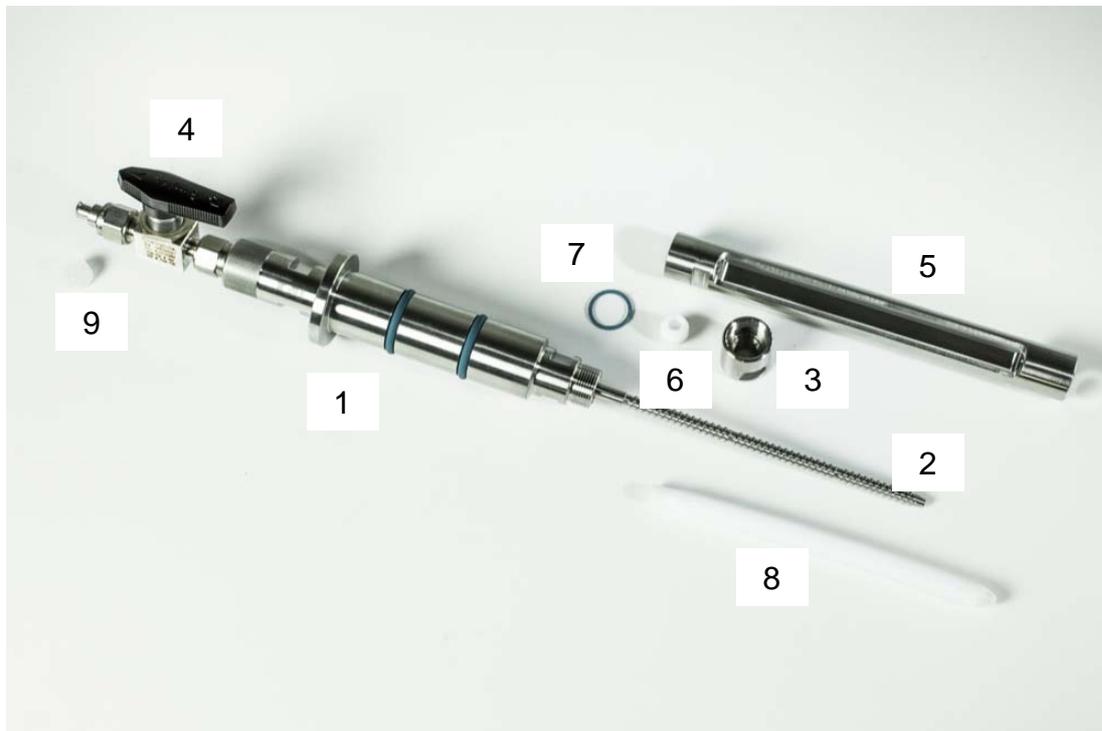


Figure 2: Parts of the filtration probe for the installation in a 25 mm side port

The membrane protective cage (5) is available for the installation in a 25 mm side port in bioreactors with high turbulence to avoid deformation of the spiral tube of the membrane mounting (2).

The spiral tube made of stainless steel supports the membrane. Permeate that is drawn through the membrane (8) is led via a helical channel to 4 bore holes. From there, the filtrate flow the shortest route up to the head of the filtration unit (1), which prevents any remixing.

A PTFE ferrule (6) surrounds the end of the membrane and the exposed portion of the membrane mounting. By tightening the cap nut (3) resp. membrane protective cage (5) into the filtration unit, the sealing ferrule distorts under pressure and securely seals the membrane end to the membrane mounting.

The thread of the filtration probe is sealed from penetrating microorganisms by an o-ring (7) and by tightening up to the limit stops.

3. Assembly of the filtration probe

Mount the o-ring over the external thread of the filtration unit. Then push the PTFE ferrule over the spiral tube (narrow opening first) up to the conical lower membrane mounting of the filtration unit (figure 3 and 4).



Figure 3 and 4: Insertion of the PTFE ferrule

Take the polypropylene membrane out of its packaging. Place it carefully onto the spiral tube until it fits into the PTFE ferrule which has already been mounted.



Figure 5: Fit the polypropylene membrane

Carefully screw the cap nut resp. membrane protective cage over the membrane and into the filtration unit.

Tighten the joint using two wrenches up to the mechanical limit stop (figure 7).



Figure 6: Mounted polypropylene membrane

Figure 7: Tighten the cap nut



Before assembling the filtration probe, please wet the external thread with demineralized water.
Screw connection of the filtration probe is tightened up to the mechanical stop.

4. Disassembly of the filtration probe

The filtration probe is disassembled by following the steps in section 3 in reverse order. In general, the PTFE ferrule has to be replaced, when opening the screws. If ferrules are re-used, proper sealing is no longer guaranteed.



The thread on the filtration unit is a lubricant free fastening thread. Repeated disassembly and reassembly can lead to metallic abrasion in the threads of the screws. Therefore before reassembly occurs, the threads of the internal and external screws must be cleaned with a non-metallic brush under running water to remove any deposits. Failure to do so could result in an uncleaned screw corroding or welding, thus preventing it from being used in the future.

5. Hydrophilization of polypropylene membrane

New membranes are impervious to aqueous media (hydrophobic).

Therefore, the membrane must be hydrophilized after mounting into the filtration probe. Use 70 % vol/vol Isopropanol as the hydrophilizing solution.

Immerse the filtration probe in a glass cylinder of sufficient depth that the liquid level completely covers the membrane. Connect a peristaltic pump to the sample outlet of the filtration probe and draw alcohol through it at a flow rate of 1 – 2 ml/min for at least 2 hours. The probe can also be hydrophilized without using a pump by soaking it overnight in isopropanol.

If you are using the filtration probe in connection with the online analyser TRACE C2 Control or BioPAT®Trace you can perform the hydrophilization and the leak test with the hydrophilization set (see user manual of the analyser).

6. Leak test

After hydrophilization a leak test should be performed. Use the hydrophilization set-up from section 5.

Instead of drawing-out isopropanol through the membrane, pump air into the filtration probe. The flow rate should be the same as it was during hydrophilization.

- Many small airbubbles should leak from the membrane delayed in time (after 6 minutes). If there are no bubbles the membrane is hydrophilized insufficient.
- Leaking of big air bubbles from the couplings indicates defective sealing.

Store the filtration probe in hydrophilizing solution (isopropanol) to prevent the need to rehydrophilize.

Directly, before installing the filtration probe into a bioreactor replace isopropanol by water to avoid evaporation and the loss of hydrophilization.

7. Installation in the bioreactor

Before installing the filtration probe into a bioreactor, check that all couplings are securely fastened and tightened. Inspect the o-ring on the filtration probe and replace it if it is damaged.

Select a port of the bioreactor which will allow the filtration probe to be placed such that the membrane surface will always be completely immersed in fluid. The filtration probe should also be placed in an area of maximum turbulence (near an impeller) to maximize the filtering capacity of the filtration probe (cross-flow-effect).

After installation, please ensure that the probe does not come into contact with moving parts (e.g. stirrer or agitator shaft) inside the bioreactor. For small bioreactor is a filtration probe with an installation length of approx. 140 mm (membrane length 90 mm) available.



The installation of the filtration probe with a membrane length of 90 mm is recommended when the standard filtration probe cannot be used because of a shortage of space. Please be informed that the membrane can be blocked because of a smaller filter surface.

The shortened filtration probe is not suitable for high cell densities.

A high hydrostatic pressure inside the bioreactor can be sufficient to provide sample flow. Using the filtration probe with an on-line HPLC we recommend a feed pump (e.g. peristaltic pump) to limit the filtrate flow to max. 1.5 ml/min. Flow rates above 1.5 ml/min can lead to shortened service life due to filter pore clogging.

Always install the filtration probe just before filling the bioreactor. The membrane should never be allowed to dry out, as that could lead to loss of its hydrophilic characteristics.

8. In-line sterilization of the filtration probe

After installation into an appropriate port, the filtration probe is sterilized along with the bioreactor. The filtration probe must be completely covered with liquid during the entire sterilization cycle. Draw filtrate from the sample outlet of the filtration probe during the heating cycle for a complete flushing of traces of alcohol from the membrane and the probe interior and for the volumes outside the bioreactor (sample connector, valves) to be rinsed. Close the shut-off cock during the sterilization cycle (typ. 125 ° C, 1.5 bar) to prevent the pressure difference which occurs at high temperatures from distorting the polypropylene membrane.

After sterilization and reaching of operation temperature inside the bioreactor (max. 40°C) the shut-off cock must be reopened, so that a connected device (e.g. online analyser, online HPLC) can collect samples.

9. Connecting the filtration probe

The filtration probe will be directly connected to the filtration tubing set of the online analyser TRACE C2 Control and BioPAT® Trace (figure 8 and 9).



Figure 8 and 9: Filtration probe with tubing set of the online analyser TRACE C2 Control / BioPAT® Trace

An installation kit (ferrule, fitting, connector, blind plug, adapter UNF/LUER) is available for the connection of the filtration probe e.g. to an external pump or the online analyser ProcessTRACE.

10. Regeneration of the filtration probe after fermentation

Immediately, after discharge and sterilization of the bioreactor, remove the filtration probe from the port. We recommend exchanging the membrane and PTFE ferrule after every fermentation.

However, in many cases reuse of the membrane (3 – 5 times) is possible after cleaning. For reuse of the probe, proceed as follows:

- Using a soft brush, remove all particulate matter from the outside of the filtration probe under flowing water.
- Clean the polypropylene membrane by drawing 0.5 N NaOH solution through the filtration probe at a rate of 2 ml/min for 4 hours using a peristaltic pump and glass cylinder configuration similar to that described in section 5.
- Rinse the filtration probe thoroughly with demineralized water.
- Store the filtration probe in hydrophilizing solution (70 % Isopropanol (vol/vol)) to prevent the need to rehydrophilize the membrane.
- If the filtering performance of the filtration probe cannot be restored by thorough cleaning, a new membrane and PTFE ferrule should be installed, hydrophilized, and leak tested before use.

11. Technical Data

Installation of the filtration probe:	Bioreactor with 25 mm side port
Types:	Standard Short version (for small bioreactors with lower installation depth)
Filtration method:	Sterile in-line filtration
Filtration probe material:	Stainless steel 1.4404
Installation depth of the filtration probe:	Standard version ca. 180 mm Short version ca. 140 mm
Membrane material:	Tubular micro-filtration polypropylene membrane
Membrane length:	Standard version ca. 130 mm Short version ca. 90 mm
Pore size of the membrane:	0.2 µm
Flow rate:	1 – 1.5 ml / minute
Membrane sealing:	PTFE ferrule
Sterilization in the bioreactor:	at 125°C and 1.5 bar
Optional spare parts:	Membrane protective cage (stainless steel 1.4404)
Installation depth of the filtration probe with membrane protective cage:	Standard version ca. 205 mm Short version ca. 165 mm
Installation kit (only for the connection of the filtration probe to an external pump or ProcessTRACE):	Ferrule, fitting, connector, blind plug, adapter UNF / LUER