POCmini - Chamber - System

...................... the smaller one compared to the POC-Chamber-System

A Chamber - System for cells in vitro - for *Perfusion*, *Open* and *Closed* cultivation.

Improved microscopes, their objectives and optical techniques now allow higher standards in microscopic analysis of living cells. In addition, heated microscope stages and climate boxes are available which allow observation of cells under stabilized temperature. The climate box is capable to hold the correct temperature and the pH-value of the medium in which the cells are being cultured by regulation of the CO$_2$. (Carl Zeiss, 37081 Göttingen, Germany; Leica, 35530 Wetzlar, Germany). This is an important step in stabilizing specific parameters for the standardization of in vitro tests.

The POCmini-Chamber-System satisfies conditions for the use of different microscopic methods for observation and analysis of living cells.

*The POCmini-Chamber-System provides 4 different applications possibilities*

"Closed" Perfusion       "Closed" Cultivation              "Open" Cultivation               "Open" Perfusion B

Perfusion adapter with
4 in- outlets also available

**External Dimensions:** Base plate 58 mm Ø (Aluminium, black anodized)

**Microscope:** Universal mounting holder M and K.

**Cultivation area**
- Cultivation on glass: Cover slip 0.17 mm (ideal refractive index) or foil (e.g. CultFoil, 0.025 mm, gas permeable).

**Observation area** (17 - 22 mm Ø)
- High resolution oil immersion objectives can be used.

**Variable height of the chamber**
- Using the *closed* chamber version the distance between the upper and lower glass is variable (1 or 2 mm).
  - Cells can also be cultivated on both cover slips.

**Perfusion**
- In the case of perfusion in the *closed* version the inner height of the chamber is 0.7 mm or less.

**The chamber is alterable**
- The chamber can be modified between open and closed cultivation, as well as the perfusion in the open and closed version of the same cells.
- The cells can be precultivated on cover slips (30 mm Ø) in Petri dishes ("35").

**Advantage**
- easy to use, rapid to assemble
- produced of non-toxic materials
- sterilization of the mounted chamber

**Accessories**
- Heating Frame or Heating Insert P
- Heatable Cover for the Heating Frame
- Heating- and Cooling Frame
- Temperature regulator
POCmini
“Closed” Cultivation System

Dimensions: 58 mmØ
Observation area: 22 mm Ø

Volume: 1 mm gasket approx. 0.4 ml
         2 mm gasket approx. 0.8 ml

Material: Base plate: Aluminium, black anodized
         Screw ring: stainless steel
         Gasket: Silicon
         Cover slip: glass, 30 x 0.17 mm
         Mounting key: Aluminium with stainless steel-pins

Characteristics: Silicon: gas permeable, dry sterilization,
                 non-toxic and “closed” again when
                 the needle is removed
                 Aluminium is an extremely good heat conductor
                 Cover slip hydrolytic class 1, borosilicate glass,
                 preferable for all microscopic procedures

Feature: * Optimum conditions for the microscopic
         observation - plane surfaces - no meniscus.
         * The chamber can be turned:
           Cultivation of cells on one or both coverslips.
         * The cells can be observed on a “upright”
           microscope using a long-distance condenser.

Sterilization: +165°C for 2 hrs. - After sterilization
the screw ring should again be tightened

changing of medium

remove the medium and introduce fresh medium slowly
(the better method)
Recommendation needles using 1 mm silicon: addition with 25 G (No. 18), exit 24 G (No. 17)
using 2 mm silicon: addition with 23 G (No. 14), exit 20 G (No. 1)

addition of nutrition medium + cells

A precultivation of cells on coverslips in Petri dishes (“60er”) is possible!

A precultivation of cells on coverslip in Petri dishes (“60er”) is possible!

 steril cover slip

A precultivation of cells on coverslip in Petri dishes (“60er”) is possible!

 A precultivation of cells on coverslip in Petri dishes (“60er”) is possible!

the different components of the POCmini-Chamber must be sterilized beforehand
POCmini

“Open” Cultivation System

Dimensions: 58 mm Ø
Observation area: 17.5 mm Ø
Volume: 1 mm gasket approx. 0.4 ml
2 mm gasket approx. 0.8 ml
Material: Base plate: Aluminium, black anodized
Screw ring: stainless steel
Gasket: Silicon
Cover slip: glass, 30 x 0.17 mm
Mounting key: Aluminium with stainless steel-pins

Characteristics:
- Silicon: gas permeable, dry sterilization, non-toxic and “closed” again when the needle is removed
- Aluminium is an extremely good heat conductor
- Cover slip: hydrolytic class 1, borosilicate glass, preferable for all microscopic procedures

Feature:
- Optimum conditions for the microscopic observation - plane surfaces - no meniscus.
- The chamber can be turned:
  - Cultivation of cells on one or both cover slips.
- The cells can be observed on a “upright” microscope using a long-distance condenser

Sterilization: +165°C for 2 hrs. - After sterilization the screw ring should again be tightened

Introduce or displace nutrition medium, cells etc. as a Petri dish.
A precultivation of cells on cover slips in Petri dishes (“35”) is possible!

sterile cover slip
cover slip with cells

the different components of the POCmini-Chamber must be sterilized beforehand

* Changing from “open” cultivation chamber (glass) to the “closed” perfusion system under the laminar air flow is possible using the 0.5 mm silicon gasket:
  - sterilize mounting key - remove the PTFE adapter - place the sterile perfusion adapter with the 20 mmØ cover slip and the silicon gasket, fix this with the mounting key

LaCon
POCmini
Perfusion in the "closed" cultivation chamber

(mounting key)
screw ring
silicon ring, 23 mm Ø
cover slip (20 mm Ø x 0.17 mm)
perfusion adapter with canal tubes

for 2 or 4 inputs/outputs

Silicon gasket 0.5 mm = 0.7 mm
FEP gasket 0.2 mm = 0.4 mm

Inner height of the chamber:

Sterilization:
+165°C for 2 hrs. - After sterilization the screw ring should again be tightened

Example of a perfusion

* Draw the sterilized tubes (e.g. silicon tube, innerØ 0.7-0.8 mm) over the canal tubes (stainless steel tube, innerØ 0.6 mm) at the perfusion adapter.
* Normally, the perfusion is realized by pressure: syringe by hand automatic syringe pump peristaltic pump
* Flow rate: For optimal physiological conditions in cell culture with a middle density a flow rate of 0.1 - 0.25 ml/hr. is used.
POCmini
Perfusion in the “open” cell cultivation system

Perfusion adapter B
screw-in, with a special flat PTFE adapter
(micromanipulation, injection of cells)

Perfusion adapter B:
The distance between the growth surface
and the top of the adapter = 7.5 mm

Sterilization:
+165°C for 2 hrs. - After sterilization
the screw ring should again be tightened

section through the perfusion adapter B

* observation area: 22 mm
* open access to the cell culture (cover)
* the medium changes can be performed either slowly
  or rapidly using two canal tubes of different length
* the height of the tubes within the PTFE adapter can be varied
  for a distinct amount of medium

not included
Example of a perfusion system

Peristaltic pump
If a multicanal peristaltic pump is used, the influx and eflux of the culture fluid can be performed by using the same pump. In this case the tube through which the fluid is removed has a slightly greater diameter than that through which it is enters.

Syringe pump
Instead of a peristaltic pump, a syringe can be used to introduce medium into the chamber.

Medium bottle
A 25 mm diameter hole is bored into the top of the medium bottle lid. A 4 mm thick silicon plate is used for sealing. A needle, for example an injection needle (1.2 mm external dia., 15 mm long) is inserted through the silicon seal and bent slightly. The needle is inserted not through the top, but through the bottom of the seal so that it will be immersed in the medium when the lid is put on the bottle. So that the pressure is equalized, a second needle is pierced through the seal. This needle is attached to a hydrophobic sterile filter. The unit without the filter must be sterilized.

Silicon tubing 1
Tubing with an internal diameter of 0.7 mm for medium influx

Silicon tubing 2
Tubing with an internal diameter of 1.0 mm for medium eflux
The outer diameter of the perfusion adapter tubes is 1.4 mm, the inner diameter is 1.0 mm.
Different types of tubing can be used;
e.g. silicon, Norprene®, Tygon®, Teflon®. See Material Description!
All components can be dry-heat sterilized (2-3 hrs. at +165°C) or autoclaved at +121°C

Perfusion Procedure
slow perfusion
Medium is passed from the medium reservoir bottle through the tubing via the pump into the POC-Chamber through the longer of the two adapter tubes. The medium is withdrawn through the shorter adapter tube.
The perfusion speed can be varied widely, but an optimal flow rate for a cell culture would be between 0.1 and 0.25 ml/hr.
For long-time observations, an inverse microscope with a constant temperature stage with the possibility for constant pH-values must be used. For these studies a climate box, in which the temperature and CO₂ is controlled, is used in conjunction with the heated microscope stage (Carl Zeiss, 37081 Göttingen; Leica, 35530 Wetzlar). The medium container can then be outside of this box. The slow perfusion allows the medium to be warmed as well as the pH to be controlled via the gas permeable silicon tubing, before it reaches the cells.

fast perfusion
To achieve a fast perfusion, the medium enters through the longer perfusion adapter tubes and exits via the shorter tube. A syringe can be used for this procedure. In this way, a medium change can occur in about 8 sec: addition of 2.5 ml in 4 sec and removal under vacuum in 4 sec. In this case, silicon tubing with an internal diameter of 0.7 mm is used. Since the internal diameter of the tubes of the perfusion ring is 1.2 mm, this allows the flow rate to be increased with a reduction in time of between 2.5 and 3 sec.
POCmini
Perfusion of Cell Culture in the POCmini

Regulation of °C and pH-value using the incubation system on the inverted microscope (Axiovert 100, 135) with scanning stage or mechanical stage

Components:
Incubator S, Heating Insert P,
CTI-Controller 3700,
Tempcontrol 37-2 digital

Special feature:
* Bypass System, in which the conditioned airstream may be diverted through a channel before the cover is removed.
* The LD-condenser 0.55 may be used.

POCmini, perfusion, observation area: 17 mm Ø

Heating Insert
#411861-9901-000
POCmini-Chamber-System on "upright" microscopes

The POCmini-Chamber-System can also be used on "upright" microscopes. In this application the working-distance of the objectives and condensers is important.

Closed cultivation

Perfusion in the closed system

The POC-Chamber can be turned
Material Description

Aluminium: Base plate and mounting key
- Aluminium is an extremely good heat conductor. The surface of these parts are heat-treated and strengthened due to an anodized coating. However, this procedure does not compatible with strong alkali cleaning materials. For this reason, these parts should only be cleaned with distilled water or 70 % ethanol.
- The black anodized base plate should not be placed into a CO₂-incubator with copper trays since this will cause “electrolysis” and damage the surface of the chamber. In this case place the chamber on a glass, plastic or stainless steel base.

V2A-Steel: Perfusion adapter, Perfusion adapter B, screw ring, cover frame and canal tube
- The Perfusion adapter for closed cultivation should be handled with care because the rim which supports the cover slip (0.2 mm, on the outer area, 0.1 mm) can easily be bent. The canal tube can be cleaned with 70 % ethanol or other cleaning fluids and well rinsed with distilled water.

PTFE und FEP: Registered name Teflon® (DuPont), Hostaflon® (Hoechst)
- PTFE adapter: This is a very inert material which is insensitive to most cleaning materials. It is best cleaned with 70 % ethanol or other laboratory cleaning fluids and then rinsed well with distilled water. temperature: PTFE more than 200°C
- FEP-Foil (CultFoil, PeCon): This foil has a treated (hydrophilic) and smooth (hydrophobic) surface. The thickness of the foil is 25 µm.

To distinguish between the two surfaces
- a.) The hydrophilic side demonstrates an adhesion to paper.
- b.) It is not possible to write on the hydrophobic side using a felt tip pen

Characteristics and uses
- high gas permeability
- permeable to ionizing radiation even below 300 nm
- non-toxic
- inert to most cleaning materials
  - hydrophilic surface:
    - excellent cell adhesion
  - hydrophobic surface:
    - suspension culture
    - colony formation in agar or methyl cellulose
    - can be easily removed from resin after embedding for electron microscopy
    - temperature: +170/180°C

Silicon: Gaskets: non-toxic, gas permeable with a hardness of 40 and 60 Shore
- After use, wash immediately under running water and rinse in distilled water. If necessary, boil for 10 min in distilled water or autoclave at +121°C.
  Do not use cleaning liquids, although ethanol can be employed.
  temperature: +180°C

Tubing material for perfusion: non-toxic
- Silicone is very elastic, gas permeable, can be sterilized by autoclaving as well as by dry heat (165-180°C).
- Norprene® is much less gas permeable, but has a longer life than silicon in a peristaltic pump. It can also be autoclaved (121°C).
- Tygon® tubing is crystal clear, does not oxidized, has very smooth surfaces, is elastic and ideal for peristaltic pumps. It is not particularly gas permeable and can be autoclaved (121°C).
- Teflon® (PTFE, TFE, FEP) is extremely inert to almost all solvents: If thin tubing is used, it is gas permeable but is not particularly elastic. It must be connected to steel tubes of the perfusion adapters by silicon tubing and cannot be used in peristaltic pumps. It can be sterilized by dry heat or autoclaving.
Glass: cover slips, 0.17 mm
- Hydrolytic class 1, borosilicate glass. Cleaning is not usually necessary. However, normal laboratory cleaning liquids can be used followed by rinsing in distilled water. Always use a pair of tweezers to handle the cover slips and grip them only on the rim.
- **Recommended cleaning in a laminar air-flow bench:** Dip the cover glass in ethanol and let the latter drip into a beaker. Then pass the cover slip through a flame to burn off rest of the ethanol. Keep the cover slip in a horizontal position until it has cooled.

Sterilization: Either the whole chamber or parts of the chamber can be sterilized.
- Dry sterilization at +165°C to 170°C for 2 h
- Autoclaving at +121°C
After sterilization of the perfusion or "closed" chamber, the screw ring should again be tightened.

**Trouble-shooting**

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass breaks when putting chamber together or by sterilization</td>
<td>Bad quality glass</td>
<td>New glass of a better quality</td>
</tr>
</tbody>
</table>

"Closed" culture:
- Too much pressure within the chamber during sterilization
  Insert a needle trough the silicon wall to release pressure inside the chamber

"Open" culture:
- Instead of the PTFE adapter for "glass", the adapter for the FEP-Foil has been used
  Change the adapter

<table>
<thead>
<tr>
<th>Chamber leaks</th>
<th>Screw ring not tight enough</th>
<th>Tighten ring with key</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Silicon ring not properly cleaned, so that dust is present on the surfaces</td>
<td>Clean silicon ring again, if necessary with ethanol</td>
</tr>
<tr>
<td></td>
<td>PTFE adapters: Deep scratches or indentations in the surface connecting with the silicon ring</td>
<td>Use a new adapter</td>
</tr>
</tbody>
</table>

**References**

POCmini-Chamber-System
(# 0730.xxx)

**Perfusion**

- **"Closed"**
  - screw ring (.005)
  - glass (.016)
  - silicon seal (.010-.011)
  - glass (.016)
  - perfusion ring (.006 + .017)
  - silicon seal (.013)
  - glass (.016)
  - base plate (.001)

- **"Open"**
  - cover (.004)
  - screw ring (.005)
  - PTFE-adapter (.003)
  - silicon seal (.009)
  - glass (.016)
  - screw cutting (.018)
  - PTFE

**Advantages**
- *small dimension (mm): Ø 58, height 5.5*
- *low volume: ideal for a low quantity of cells*
- *option: Perfusion adapter with 4 inputs/outputs*
- *POCmini is also suitable for the new Heating Universal Mounting Frame M-H, A-H and K-H*

**Accessories**
- cover glasses
- "closed" perfusion: 30 + 20 mm Ø
- "closed + "open": 30 mmØ
- mounting key

**Dimension (mm):** Ø 58, height 5.5

**Observation area (mm):**
- "closed perfusion" 17
- "closed + open" 22

**Cell Culture + Microscopy**
Model ‘11’ Plus

Syringe Pump

User’s Manual

Single Syringe Pump 70-2208
Dual Syringe Pump 70-2209
Single Syringe Pump with Serial Communication 70-2211
Dual Syringe Pump with Serial Communication 70-2212
## Appendix B: Syringe Inside Diameter

### Stainless Steel

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<td>30 cc</td>
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<td>50/60 cc</td>
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### Air Tite “All Plastic”

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<td>10 cc</td>
<td>15.90</td>
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<td>20 cc</td>
<td>20.05</td>
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<td>30 cc</td>
<td>22.50</td>
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<tr>
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### S GE Scientific Glass Engineering

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MINIPULS Evolution®
Laboratory Peristaltic Pump