LUDIN CHAMBER, TYPES 1, 3 & 4

FEATURES

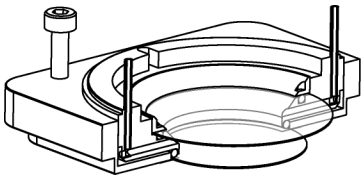
The Ludin Chamber is designed for high-resolution imaging of living cells (as well as tissues and small organisms) on inverted microscopes. It combines excellent optical properties and easy handling for a wide range of applications.

Open, closed, and covered configurations	Suitable for a wide range of applications
Made of stainless steel	High stiffness, extension coefficient close to that of coverslip → increased focus stability Sterilization with 70% ethanol or by wet autoclaving
High-precision chamber holder	Z-position of chamber is kept stably (even with rapid Z-scanning)
Width of coverslip-retaining rim 1mm only	Large viewing window relative to coverslip size
Thickness of coverslip-retaining rim 50µm only	Entire window is accessible, even when using objectives with very short working distance (**!**)
Coverslip diameter 18mm	Efficient specimen preparation possible using standard 12-well {24-well} cell culture plates
No tools required to open and close	Rapid exchange of specimen coverslip Easy handling
Internally beveled at 45°	No vignetting in transmission mode (with mid-range or long-range condensers) Good accessibility for microinjection and micromanipulation
Footprint 40x40mm only → up to six chambers fit the area of a micro-`titer plate	Enables parallel imaging of several specimen using a motorized stage and a 6-slot chamber holder
Chamber holder allows for removal and re-inserting in an accurately defined position	Carry out long-term experiments w/o blocking the microscope for others
Minimized plastic surface (O-rings) exposed to culture medium	High biocompatibility, low adsorption of hydrophobic compounds → well suited for quantitative compound testing
Chamber volume approx. 1 ml (closed configuration)	Perform short- and mid-term experiments without the need for perfusion
Built-in perfusion lines for liquids	Easily apply perfusion as required
Lid with gas perfusion lines	Perform experiments under defined atmosphere with low gas consumption

The chamber does not feature temperature control. We recommend our Cube&Box temperature control system for this purpose.

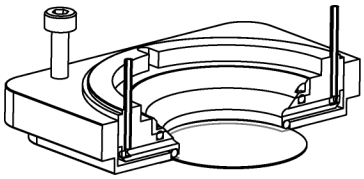
CONFIGURATIONS

Closed Configuration



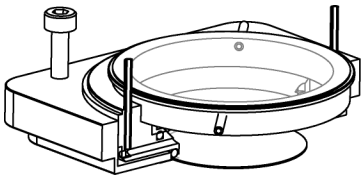
The closed configuration prevents evaporation and loss of CO_2 from the culture medium and thus obviates regulation of humidity and atmosphere. It further protects the cells from contamination and prevents liquid spills. Because of these advantages, the closed configuration is recommendable for applications not requiring direct access to the cells. For long-term experiments, the perfusion lines can be used to refresh the culture medium.

Open Configuration

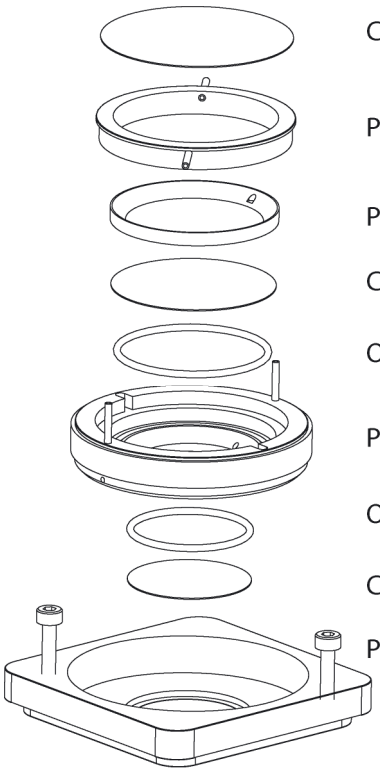


The open configuration is used for applications requiring constant access to the cells (e.g. electrophysiology). Note that it may be necessary to balance evaporation and the loss of CO_2 depending on the application and the duration of the experiment. This can be achieved by perfusing fresh culture medium and/or by using a gas- and humidity control system (available as an option to our Cube&Box temperature control system).

Covered Configuration



For applications requiring only short-term access to the cells (e.g. for microinjection), the chamber can be covered during the remaining time with the lid in order to minimize evaporation and the danger of contamination. The built-in gas perfusion lines enable experiments under defined atmosphere with very low gas consumption..



OPERATING INSTRUCTIONS

General Remarks

Take care not to damage the thin coverslip-retaining lamella in Part 1 as this may compromise the sealing properties of the chambers. When screwing Part 2 into Part 1 or Part 3 into Part 2 respectively, pay attention that the parts are correctly seated. They should turn easily except for the last revolution. If not, the parts are probably tilted or (or the threads dirty) and there is a danger of damaging the threads when force is applied inappropriately.

Preparations

Before using the chamber for the first time, it should be cleaned (see section “Cleaning”).

Insert an O-ring, 20.0x1.0mm, into the groove in Part 2.

Place an O-Ring, 16.0x1.0mm {10.0x1.0mm, 22.0x1.0mm}[1], on a flat surface and pick it up with Part 2.

⁄⁄Tip: The smaller O-ring is only weakly held. It can be mounted more permanently by first distributing a small amount of cyanoacrylate glue around the lip of Part 2 (e.g. with the tip of a toothpick). Remove glue remnants with acetone before mounting a new O-ring.

It is advisable to coat the O-rings lightly with silicone grease in order to ensure good sealing. O-rings made of FPM (Viton®) or silicone rubber with Shore hardness 70 or smaller are recommended.

Fasten a 28mm coverslip to Part 4 (lid), e.g. with silicone grease.

Reconfiguring the Chamber

Change to closed configuration by unscrewing Part 3 using the reconfiguration tool, inserting a coverslip (23.5mm diam.) into part 2, and fixing it with Part 3.

Change to open configuration by removing the upper coverslip in analogy to the procedure outlined above.

Change from open to covered configuration simply by putting on the lid (Part 4. This can be done during an ongoing experiment as well.

Mounting and Exchanging the Specimen Coverslip

Use coverslips with a diameter between 17.9 and 18.2mm {11.9 and 12.2mm, 21.9 and 22.2mm}. The use of corrected coverslips with a thickness of 0.17mm is recommended for optimal results.

Use tweezers to place the specimen coverslip in Part 1. Then close the chamber by screwing in Part 2.

Upon opening the chamber, the specimen coverslip usually sticks lightly to Part 2 and can be easily removed using tweezers. If it remains lying in Part 1, raise it slightly by pushing from below until it can be seized with tweezers from above.

Note: you may hold Part 2 may at the base of the perfusion lines to screw it in or out. However, if force is required, use the reconfiguration tool in order to avoid bending or breaking the perfusion lines.

⁄⁄Tip: Clean the lower surface of the coverslip with distilled water (and possibly ethanol) and wipe it dry afterwards, in order to avoid contamination of the immersion medium and the objective by culture medium sticking to the coverslip.

Filling und Perfusion

Tubing with 0.9mm inner diameter made of silicone (Tygon®) or PTFE is recommended to connect the perfusion lines.

To fill the chamber in the closed configuration, connect a syringe to a perfusion line using a piece of tubing. Hold the chamber vertically during filling such that air can escape through the second perfusion line in order to avoid bubble formation.

If longer-term experiments are to be performed in the closed configuration without perfusion, it is advisable to seal the perfusion lines (e.g. using a small piece of paraffin) in order to prevent evaporation.

Perfusion of the chamber in the closed configuration can be performed using gravity flow or a pulse-free pump.

Perfusion of the chamber in the closed or open configurations can be performed with a pulse-free 2-channel pump. It is important that the pump works precisely in symmetry to keep the chamber from drying out or overflowing.

[1] Specifications in curly braces {} pertain to Ludin Chamber Types 3 and 4 respectively

In the open configuration, perfusion can also be achieved by supplying medium by gravity flow or by a pump, and by removing excess medium from the surface by suction (an optional holder for a suction capillary is available)

⁄⁄Tip: When using passive perfusion in closed configuration, the difference in height between reservoir and chamber should be similar to that between chamber and the end of outlet tubing to keep the pressure in the chamber balanced.

⁄⁄Tip: Droplet formation at the end of the outlet tubing generates an oscillating backpressure due to the change in surface tension. In the closed configuration, this can result in focus instability owing to bulging of the coverslip. This can be avoided by immersing the end of the outlet tubing in liquid.

Cleaning and Sterilization

Distilled water and, if necessary, non-scrubbing cleaning agents suitable for stainless steel can be used to clean the chamber.

Remember to flush the perfusion lines with distilled water and, if desired, with ethanol after each use of the chamber. Do not use ethanol first, as this may result in precipitation of salts and proteins which can clog the perfusion lines.

The chamber can be sterilized using 70% ethanol. Wet autoclaving at 121°C is possible also.

If the coverslip breaks in the chamber, remove any remaining fragments from the coverslip retaining rim and the threads or leakage and damage may occur.

Care and Storage

It is advisable to treat the O-rings lightly with silicone grease from time to time in order to ensure perfect seal. Otherwise the chamber does not need special care.

Stainless steel has a certain tendency to self-weld. Therefore the chamber should be disassembled for long-term storage.