MS-OPC Instructions

Oocyte Perfusion Chamber from ALA Scientific Instruments

Introduction

The coversilp clamp dish represents a unique concept in experimental chamber design. It allows the user to constantly replace the bottom of the dish while leaving the rest of the dish intact. This way, cells can be cultured on coverslip glass (also called coverglass) and then the coverslip can be incorporated into the chamber at the time of the experiment. The chamber, which can be quite elaborate, does not need to be occupied for days in the incubator itself.

Most of the MS series chambers are made from DuPont Delrin™. Delrin is a polycarbonate plastic but it is not clear and somewhat more hydrophobic than Lexan™ (GE). In fact, Delrin is easier to machine and its black color makes it more optically friendly than Lexan™. The size and shape of the chamber match the dimensions of the Corning 35mm petri dish. The chamber can fit wherever a Corning 35mm dish can fit, i.e. a temperature control chamber, etc.

Chamber Types

ALA Scientific Instruments has a complete line of chambers based on the securing ring design. The following is a list of part numbers and the descriptions of the different chambers available:

Key code:

S = stainless steel securing ring; **D** = Delrin securing ring; **P** = 2 perfusion ports (180° apart)
T = thermal foil attached to stainless steel securing ring (25.5Ω, 0.6”/15.24mm hole);
W = chamber walls removed;

MS-502 = chamber with ¾”/19mm center hole used with 24/25mm coverglass
MS-508 = chamber with ½”/12.7mm center hole used with 18mm coverglass
MS-518 = chamber with oval center hole used with 24/25mm coverglass

Please note: whenever a **T** is in the part number the securing ring used will be a ½"/12.7mm center hole stainless steel type. This type of securing ring best supports the dimensions of the thermal foil. Any other securing ring will obstruct the viewing area. Also, Delrin securing rings can not be used with the thermal foil.

The different chamber options are:

MS-502S; MS-502SP; MS-502SPT; MS-502SW; MS-502SWT; MS-502SPWT
MS-502D; MS-502DP; MS-502DW; MS-502DWP
MS-518S; MS-518SP; MS-518SPT; MS-518SW; MS-518SWT; MS-518SPWT
MS-518D; MS-518DP; MS-518DW; MS-518DWP
MS-508S; MS-508SP; MS-508SPT; MS-508SW; MS-508SWT; MS-508SPWT
MS-508D; MS-508DP; MS-508DW; MS-508DWP; MS-OPC-REF

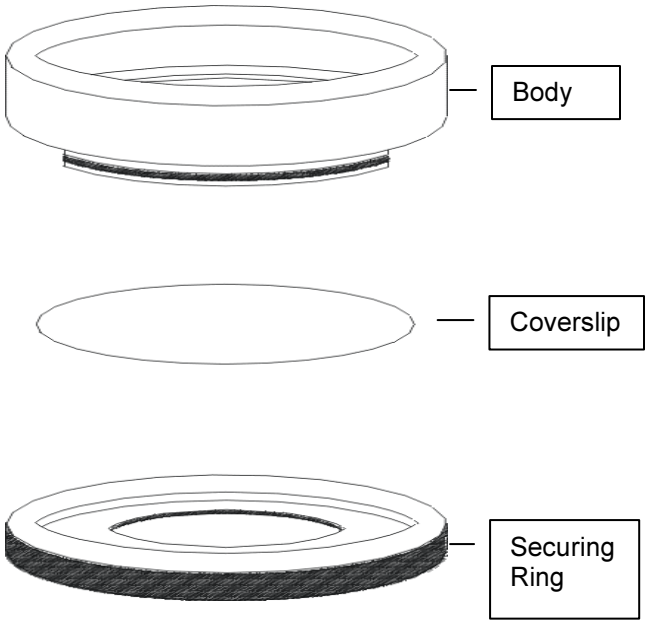
Please use the above key codes to find the correct part number.

Materials

All chambers that are black in color are made of DuPont Delrin™. Chambers can be made out of Lexan™ (GE) by special request. Delrin™ is preferred since it is opaque to light (black in color). Securing rings can be made out of stainless steel or Delrin™. Stainless steel is recommended. Stainless steel is also necessary for temperature control applications.

Setup

A coverslip clamp chamber is easy to assemble. First place the Securing Ring on a firm level surface with the lip upward. Place the cover-slip, cell-side-up, onto the ring, being careful to center it. Push the body of the dish into the securing ring by applying firm pressure. The sealing o-ring will be compressed against the glass and form a water tight seal. The securing o-ring holds the Securing Ring in place by friction. When applying firm pressure to the assembly, be sure to push down evenly all around. The chamber should now have a leak free seal.



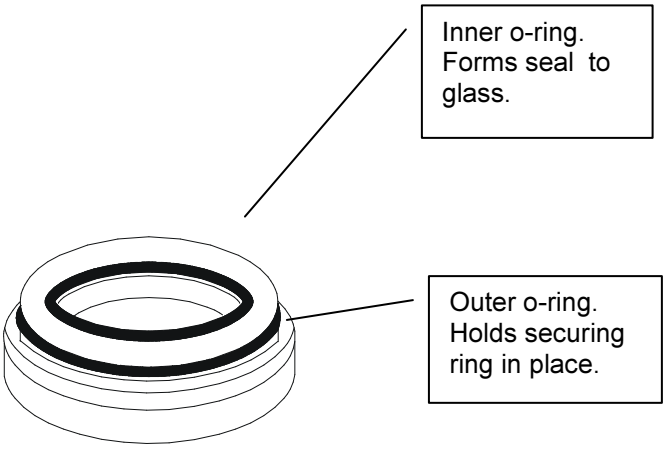
To open the chamber, simply pull the securing ring off. If it is difficult, you can use a small tool such as a screwdriver, just be careful not to damage the chamber. The chambers are designed to be opened by hand and this is the preferred method of opening.

Care and Cleaning

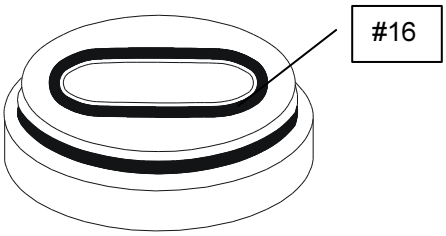
Chambers should never be cleaned with petroleum products. Regular dishwashing soap is ok, and diluted bleach solutions can be used. A thorough cleaning should include removal of the o-rings to clean the o-ring slots as well. The chambers can be cleaned in a dish washer. Autoclaving is also possible, but not recommended since high heat can warp and degrade plastics. For cleaning and sterilization, use as low a temperature as possible and never exceed 110°C. Always be sure chambers are clean and dry when they are packed for storage.

The stainless steel securing ring is made from 316 stainless steel. It is amongst the most durable steel available. However, the use of saline solutions can affect almost all steels. After all, it is called “stainless” not “stain proof” or “stain free,” so some pitting or staining is possible after a while. Try to keep the steel securing rings clean and dry.

Cover slip clamp dishes are designed to be used with #1 or #2 round coverglass or coverslips. Glass thickness ‘0’ is generally too fragile to be used. Most dishes are made to be used with 25mm diameter glass, but some dishes are built for 18mm glass at the customer’s request. From the size of the inner o-ring it is easy to tell which size glass is required for your dish.



Round shaped chamber



Oval shaped chamber

O-ring replacement guide:

Standard round chambers for 25mm glass: Inner o-ring = #19, Outer 0-ring = #21

Note: Outer o-ring for all chambers is #21

Inner o-ring for 18mm glass chamber is #16.
Inner o-ring for oval shaped chamber is #16.

(O-rings are generally made of Silicone, but Viton is available upon request.)

Replacement o-rings and parts are available from your distributor or ALA. (*www.alascience.com*)

Troubleshoot

Leaks

The coverslip clamp dish stays closed by the friction of the securing ring to the outer o-ring. The outer o-ring does not help seal in the liquid. Only the inner o-ring seals the liquid in. If the chamber leaks it can be for several reasons:

Problem	**Solution**
1) Inner o-ring is not seated.	Press inner o-ring into groove.
2) Outer o-ring does not grip securing ring.	Replace outer o-ring, replace securing ring.
3) Cracked glass.	Open chamber, inspect/replace glass.
4) Nicked inner o-ring.	Replace inner o-ring.
5) Warped dish.	Refer to factory.
6) Warped securing ring.	Refer to factory.
7) Particles trapped on or under o-rings.	Remove/clean o-rings and groove.

OPC Specific Instructions

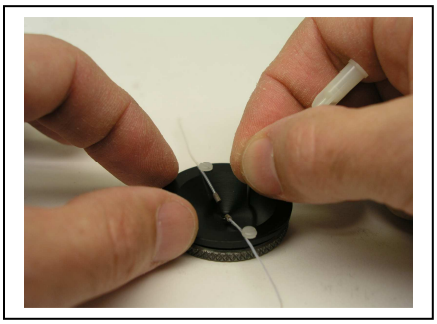
The OPC is a unique chamber that uses a funnel effect to channel perfusion solutions around an Oocyte in a very efficient manor. The Oocyte is placed at the bottom of the funnel, on the glass surface of the cover slip that forms the bottom of the chamber. Typically the Oocyte will adhere to the glass to some extent. The position of the Oocyte at the bottom of the funnel insures that it will be well bathed by the perfusion solution that is put into the chamber through the metal canula that is provided (19gage). Typically the canula is the infusion point, but it can be used in reverse, if a device such as an ALA Levelock™ is used to manage the solution outflow. If your suction line is simply tied to a vacuum source, then the canula should be used for infusion, and your suction line placed on the side of the funnel. (If you require adjustable holders for tubing with a magnetic base that can secure the chamber and the tools, please check out MS Tools from ALA scientific as well)

The funnel shape of the chamber helps to keep fluid levels constant by allowing an ever increasing space as the fluid rises. Also, the shape gives solutions space to spread out and dilute after application on the Oocyte when the next solution is infused at the bottom. In addition, the small volume created by the chamber at the bottom insures fast perfusion time, and the shape of the walls will give maximum axis for two recording electrodes.

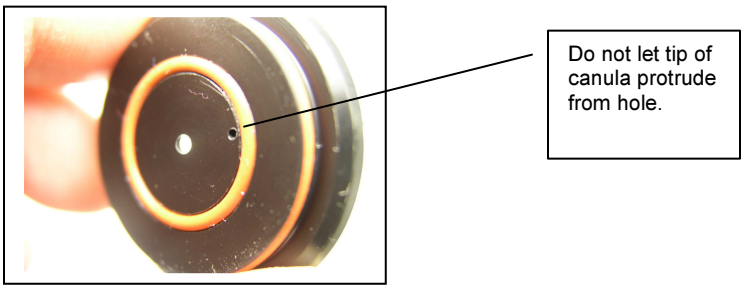
Set-Up

When your OPC arrives, if you ordered it with pellets (Ag/Cl reference electrodes), they will be installed as such:

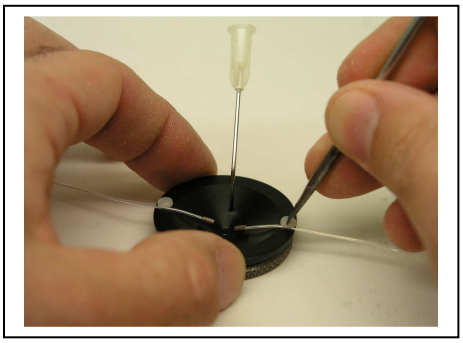
And there will be an infusion canula. The canula is installed by holding the chamber down on a flat surface and carefully inserting the tip.



As you are inserting it, be careful not to let the tip protrude out of the bottom, or the chamber will not be able to be sealed:

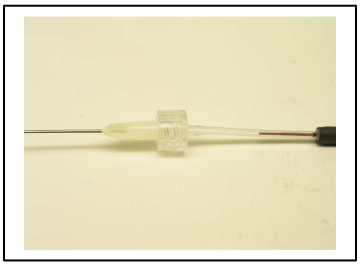


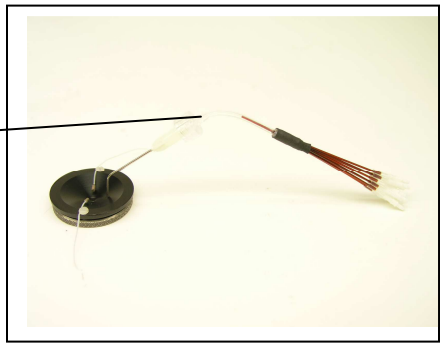
If you need to adjust the position of the Silver Chloride pellets, or install them, use a small screw driver to loosen or tighten the plastic screws on either side of the chamber. Remember that the wire must pass through a small groove under each screw to be properly held in place.



To connect the chamber to a perfusion device such as the ALA-VC3-8 with a Millimanifold™ as the output, you will need a small adapter. The Luer-MLF adapter is available from ALA, it simply consists of a 2.5cm length of 1mmID silicone tubing placed on a male Luer fitting with a small barb for the 1mm ID tubing.

Luer-MLF adapter





The adapter will allow the Millimanifold™ to be connected to the infusion canula with little difficulty. Removal is easy with just a twist of the Luer.

Also, please note that the infusion canula can be bent more if necessary to be more out of the way of optics or manipulators. Just be sure not to bend the very tip (Last centimeter) to be sure it can still be inserted in the chamber. Lastly, avoid constant inserting and removal of the canula from the chamber as it will tend to enlarge the hole after a while, and loosen the fit of the canula.

Additional information can be gotten by emailing support@alascience.com, or phoning 631-393-6401 in the USA.