Model # MS-CPC
INSTRUCTION MANUAL

ALA Scientific Instruments Inc.
60 Marine street
Farmingdale, NY 11735
Tel. # 631 393-6401
Fax: # 631 393-6407
E-mail: support@alascience.com
www.alascience.com

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Description

The Perforated O-Ring Closed Cellular Perfusion Chamber is designed for use in cell biochemistry, physiology, biophysics and imaging research. It holds a sample of living cellular material on a glass coverslip forming a sealed chamber with another glass coverslip. (Cells are either cultured on the glass, or deposited there prior to closure of the chamber.) The two coverslips are separated by an o-ring made from an elastomeric material such as Buna-n or Viton. When the chamber is closed, the o-ring separates the two pieces of glass and defines the actual "space" of the chamber. The O-ring is perforated in several places enabling a tube to be inserted through one perforation to provide an inlet for perfusion. The fluid moves out of the space defined by the o-ring and the two coverslips through other perforations in the o-ring. Perforations are made with a pointy object such as a needle. The effluent can either flow out through another tube passed through the o-ring, or seep out through the holes in the o-ring. The effluent that emerges from the o-ring is collected in the space surrounding the o-ring but within the confines of the nest and the top ring. The effluent can be removed from this space with a small suction tube.

Dimensions

35mm x 7mm Approx.

O-ring: Standard is 12mm ID x 1.75mm high. (Square profile.)

Unit works best with 25mm round cover glass. Other sizes can be substituted so long as viewing hole supports the size. (Specifications subject to change.)

Photo of chamber with PE-10 tubing inserted.

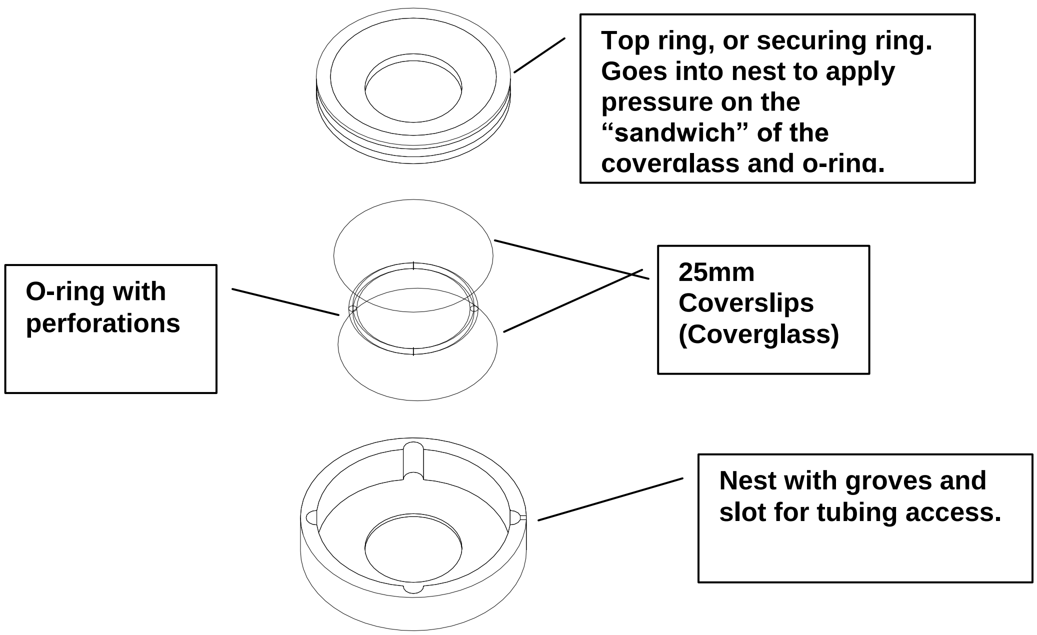
The chamber consists of the following items: First, a nest into which all the parts fit into. This nest is designed to match the dimensions of a 35mm Petri dish as close as possible. The next item into the nest is a 25mm cover glass, then the o-ring, then another cover glass and then the top ring. The top ring is pushed down into the nest to clamp down the other parts. The top ring has an o-ring at the periphery that holds it in the nest by friction with the inside wall of the nest. The securing ring (top ring) is made from Stainless Steel or Delrin® (Dupont) and may be cut or tapered to enable better microscope objective lens access. The nest is typically made from stainless steel. It has certain groves and cuts to allow access for tubing and disassembly tools. All holes, dimensions and o-ring sizes can be varied at the customer's request.

In addition, a thermofoil can be added to the bottom of the nest to provide a heat source for temperature control of the chamber. A thermistor or other temperature sensor can be placed in the inner or outer part of the chamber. Placement in the inner part would be by passing it through a perforation in the o-ring.

Instructions for use

Below is an explosion drawing showing the assembly of the chamber:

Explosion Drawing - Perforated O-Ring Closed Cellular Perfusion Chamber



Note: A pointy (not sharp) tool may be used to open the chamber by prying at the top ring through the groves in the nest.

The chamber is formed from several parts that must be prepared and assembled carefully for the chamber to work correctly. The most important component is the perforated o-ring. The standard one supplied is made from Buna. It is a soft synthetic rubber that is impervious to most substances of biological origin. The thickness of the o-ring determines the spacing between the two cover glasses and thus determines the volume and separation of the glasses. The MS-CPC uses a special o-ring that has a square profile. As such, it has four sealing surfaces, and remains in position (can't roll like a round o-ring) so it is like a rigid member of the chamber even though it is soft. This allows the ring to be punctured in various ways and to retain the holes that form in the proper orientation.

In order to get fluid into the chamber, a tube must carry fluid in. This means that it must pass through the o-ring. We recommend PE-10 tubing, which is a soft polyethelene tube such as that used for epidural catheters. The procedure is very similar. A hollow injection needle with ID large enough to accommodate the PE tube (about 19GA for PE-10) is pushed through the side of the o-ring in the groove. When the needle protrudes to the inside, the PE-10 tube is pushed through to the inside of the 0-ring. The needle is withdrawn, leaving the tube piercing the o-ring. The tube is withdrawn until just the tip of the tube can be seen on the inside of the o-ring. The length of the PE tube can be shortened to 10cm or less. Since it has such a small bore, it is often advisable to mate it to a larger bore tube to increase flow rate.

Instructions for use (cont.)

Fluid can leave the chamber in three ways. If you do not compress the chamber assembly too hard, fluid can leak out between the o-ring and the surface of the glass coverslips. Fluid can get out through holes that you puncture in the o-ring, and fluid can emerge through an additional tube at another part of the o-ring. (This may require a small modification of the chamber nest to add another slot.)

The most favored exit style is through punctures in the o-ring. These are made with a sharp needle. This is where the perforated o-ring system gives the user a great advantage. Although the input is limited to one or two locations where the PE tube can get in, the output can be anywhere and at any number of locations. This helps to assure good fluid flow through the chamber and gives the user total control of the flow pattern in the chamber. Thus it is important to consider what flow pattern you would like to achieve and make the appropriate punctures in the o-ring before assembly. (The chamber is supplied with an o-ring with a PE-10 tube inserted, and three drainage punctures at 90° intervals around the o-ring.

When the chamber is being used, fluid that leaves the cell space will go into the space in the nest surrounding the cell space. Fluid will accumulate in this area. It should be removed with a suction tube that is inserted into one of the grooves. The suction tube should have constant suction since this will not disturb the cell preparation at all and prevent flooding. (We recommend the ALA-VWK Vacuum Waste Kit for this purpose)

Chamber Assembly

1) Place the bottom (nest) in a flat rigid surface.
2) Place the bottom cover glass in the nest centered over the hole. This can be tricky if cells are on the cover glass so use precaution to keep them wet and comfortable.
3) Place the o-ring on top of the bottom cover glass. Any tubing inserted through the o-ring should be placed in the slot of the nest. (This is where soft tubing like PE seems to be essential since the stiffness of the tube can upset the o-ring.) Careful technique and patience is important here. (Users may prefer Teflon® (Dupont) tubing, which we can supply, but it may make this assemble more difficult do to the natural stiffness of Teflon tubing.) After the o-ring is set, more fluid can be added to bathe the cells and prime the chamber before it is closed.
4) Place the top cover glass on the o-ring taking care to center it as much as possible.
5) Place the top ring of the chamber on top of the nest and carefully and evenly push it down into the nest until it compresses the entire assembly. (To establish a leaky chamber, if desired, do not push down to hard, but make sure top ring is even, not tilted. Do not pull on the PE tube as his might displace the o-ring.
6) Begin perfusion immediate since live tissue will require oxygen and nutrients to remain viable. See that all air bubbles are pushed out.
7) At the end of experiment chamber should be placed on rigid surface and opened using a pointy (not sharp) tool to pry open the top ring. Disassemble carefully and clean off all salt solutions.

Chamber Assembly (cont.)

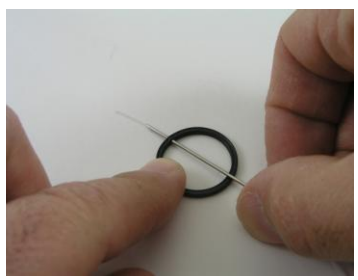
It is easier to assemble the chamber when the cover glass has a larger OD than the o-ring supplied.

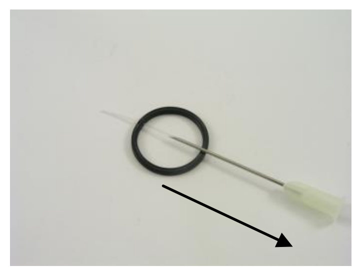
Precautions should be taken to prevent air bubble entry into the chamber. If a bubble forms, it should be easy to pass through one of the perforation in the o-ring. It might be necessary to lift the chamber and hold it at an angle to force the bubble out.

If you have a thermofoil mounted on your chamber, it must be connected to the temperature controller. Before you place cells in the nest, the nest must be warmed to insure no trauma to living tissue. For customers using temperature control equipment we supply mini thermistors that are inserted into the chamber through the o-ring like the PE tubing. This may require an additional slot in the nest and should be ordered as such, but it can be added later simply by sending your chamber to ALA Scientific Instruments. It should be noted that ALA can supply complete temperature control equipment to regulate both the temperature of the chamber and the incoming fluid.

Fluid may be provided to the chamber from a syringe pump, peristaltic pump or other gravity means. Flow rates must be determined empirically to insure proper cell adhesion, minimal disturbance, low-pressure gradient, accurate temperature control where necessary, and of course cell viability. Remember that in order to accommodate high flow rates, additional holes in the o-ring might be necessary.

O-Ring Perforations:





Pierce the o-ring with a 19GA needle, insert PE-10 tubing through needle.

Withdraw needle so that tubing remains passing through O-ring.

Withdraw PE-10 tube until end is just inside o-ring. Place Luer compression fitting on tube. Additional holes or cuts can be made in o-ring to facilitate fluid evacuation from the chamber. Short lengths of tubing may be used as outflow ports as well.

Cleaning and Sterilization

Unit may be placed in a dishwasher. All o-rings should be removed, washed and replaced. Heat sterilization for o-rings is not recommended but is possible up to 110°C. Alcohol may be used for cleaning. PE tubing should be purchased sterile if necessary.

Warranty

ALA Scientific Instruments agrees to warranty this instrument for a period of one year from date of shipment. The warranty covers all parts and labor necessary to correct defect(s). ALA will, at their option, repair or replace nonworking components. ALA is not responsible for damage resulting from the improper use of this product. User acknowledges that this chamber is not designed to retain fluid and that fluids must be actively removed while this device is in use. ALA is not responsible for damage to other equipment from fluid that leaks or spills from this device.

Installation of this system in a manner inconsistent with this manual will render this warranty null and void. No other warranties are expressed or implied. Your rights may vary from state to state.