

Scientific Systems Design Inc

Innovative Engineering for Science

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Microscope Stage Cover Slip Chamber

Submerged Preparations



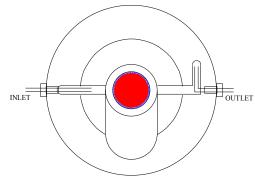
The MS-5 chamber has been designed for use on microscope stages to view glass cover slips containing cultured cells. Cover slips smaller than 24mm with cell cultures are placed directly on top of a fixed glass cover slip glass at the base of the MS5. The chamber can be used with inverted microscope stages but the glass cover slip at the base of MS5 will add 200um to the existing cultured cover slip thickness. The glass-to-glass adhesion between the cover slips keeps the sample stable for viewing under submerged conditions. Incubating media flows across the cultured cells with the possibility of temperature control with an in-line perfusion solution heater MH02 available separately. The particular feature of this chamber is the raised glass cover slip that allows the cover slip containing cultured

preparations to be slipped off side-ways and returned to the incubator if required. Adaptor plates are available for various microscope stages.

FEATURES

- * Approx 2 ml capacity flow through microscope stage chamber
- * Unique design for supporting cover slips from incubated preparations
- * Glass cover slip base
- * Easy chamber removal for cleaning
- * In-line perfusion solution temperature control unit available

VIEW FROM ABOVE



22mm glass coverslip forming base of chamber



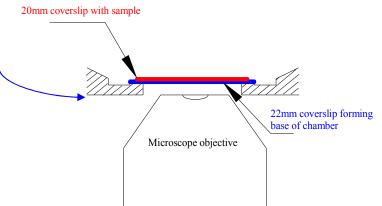
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SPECIFICATIONS Size: 100mm diameter X 10mm Material: Clear acrylic Coverslip base: 22mm X 150um . Inlet tube: Stainless steel tube, 1.8 mm O.D. Exit tube: As for inlet tube Dead space: Min. capacity 2 ml.



Perfusion solution enters from the inlet via a stainless steel tube and exits through an 'L' shaped well in which a movable tube attached to a pump / suction line adjusts the fluid level. Solutions can be pre-warmed before entering the chamber with our Mini Heating Module **MH02**. A recessed area allows cover slips containing samples to be slid off and replaced with a fresh preparation. Perfusion solution forms a pool around the preparation whilst being refreshed by virtue of the inlet and exit ports.

VIEW IN CROSS-SECTION





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MS5

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FREQUENTLY ASKED QUESTIONS

My perfusion solution forms large globules around the center of the chamber and has difficulty forming a continuous inflow-outflow stream, why does this happen?

This will happen with a new chamber or one that has just been thoroughly cleaned. The polished surfaces of acrylic are hydrophobic, so solutions tend to form globules rather than forming a curved meniscus with the walls and channels of the chamber. To overcome this problem for a new chamber or one that has been thoroughly cleaned, leave a saline (not ACSF) solution to form a pool in the chamber overnight. Rinse as normal before use for the next experiment. We believe that small amounts of salt adhere to the surface thus allowing the meniscus to form. An alternative approach is to cut strips of lens tissue 5mm in width and lay these along the inlet and exit channels to encourage flow across. In some situations it may be necessary to place a piece of lens tissue at the mouth of the exit tube to encourage smooth outflow if the peristaltic pump is too pulsatile.

How do you hold slices down?

The most reliable method is to prepare a U-shaped piece of stainless steel wire, which is then partially flattened to form a suitable surface for adhesive. Thin strands of nylon such as those obtained by separating out the individual fibres in a length of string from dental floss are positioned taught across the U-shape. Cyanoacrylate adhesive is then applied to the flattened surface on which the fine nylon threads are held taught. Once completely dry the excess ends of the fibres are cut. This "harp" is then sunk over the submerged slice preparation. We are hoping to manufacture these at some point.

What is the best method of cleaning the chamber after use?

Rinse with plenty of distilled water and leave dry before the next experiment. NEVER use a solvent of any kind as acrylics tend to fragment and can be completely destroyed. A mild acid (citric) should be used to get rid of deposits, and then left in a large volume of distilled water overnight.

How do I replace the cover slip if it should break?

Wear safety goggles for this procedure. First place the chamber on a flat, soft surface (cork tile) and GENTLY remove all the broken glass, using a small flat ended spatula in a scraping action to remove the old silicone rubber sealant. DO NOT use any kind of solvent to remove the sealant. Once completely clear, use a syringe loaded with silicone sealant and bead this around the circumference of the 18mm hole. Place a clean no. 2 thickness, 22mm cover slip on the fresh sealant and GENTLY press around the circumference ONLY of the cover slip. It is preferable to use a black sealant as it allows the thickness of the sealant to be controlled: darker areas have too much sealant under the cover slip. Try to ensure the final position of the cover slip is central. The cover slip supplied with the chamber is around 150-200um, giving room for a thin thickness of sealant. Remove excess sealant whilst still wet, taking care not to displace the cover slip. Alternatively return the chamber to us and we will replace the cover slip, please contact us for the cost of this service and further details.

Where is the best electrical ground point?

This will have to be found by trial and error. In addition to the Ag/AgCl type wire electrode you can ground the original stainless steel inlet and outlet tube to help to eliminate any artefacts from the suction line. Quite often it helps to push a grounding wire INTO the wall of silicone rubber tubes that you use for connection to your perfusion solutions, either or both the input and exit lines.