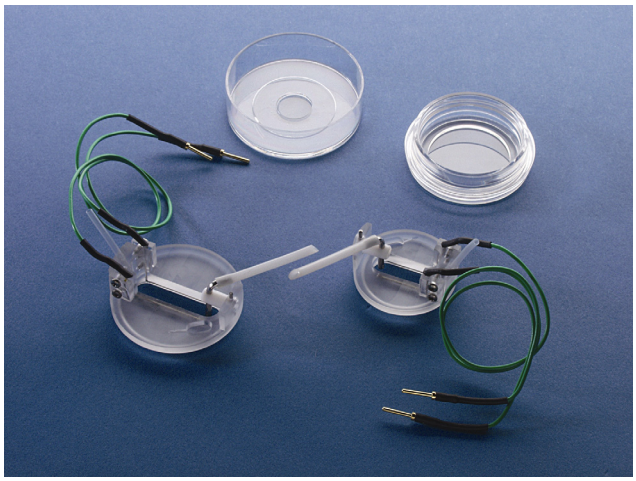
Culture Dish Chamber Instructions

RC-37WS / RC-37FS

Open Bath Chambers with Field Stimulation



A feature in common with the **RC-37 Series** Culture Dish Inserts is the use of a glass bottomed, 35 mm culture dish for the floor of the chamber. In most cases, this same coverslip contains the imaging sample. When viewed with inverted microscopes, images are visualized through a single thickness of glass, usually 0.13-0.17 mm.

The **RC-37WS** and **RC-37FS** Culture Dish Inserts are small volume imaging chambers featuring rapid solution exchange, short working distances, field stimulation, and an open bath. These chambers are designed to be securely inserted into a 35 mm culture dish allowing a variety of assays to be performed on cultured cells.

The **RC-37WS** is designed for use with Willco Wells (D35522P and D3522B) and Corning (25000) 35 mm culture dishes. The **RC-37FS** is designed for use with BD/Falcon (35-4077) 35 mm culture dishes.

Both chambers incorporate field stimulation electrodes along the sides of the bath chamber. The oval-shaped bath maintains uniform separation between the electrodes while facilitating a laminar flow through the chamber. Since bath volumes are generally small, exchange times are measured in seconds even when flow rates are less than 1 ml/min.

ASSEMBLY

A general procedure for the assembly of the **RC-37WS** and **RC-37FS** chambers is to first connect the flow lines, then fix the assembly into the culture dish. The culture dish can then be mounted to your microscope in the usual manner. Chambers are supplied with 3 m of PE-160 tubing.

Application of vacuum grease

Vacuum grease can be applied to Warner chambers by use of a syringe or a small artist's dotting brush. Both approaches are described below.

NOTE: Prior to beginning assembly make sure all required components are available and thoroughly cleaned. Be sure to remove any old vacuum grease from the perfusion channels and input/output ports.

Syringe technique

1. Begin by loading a 1cc syringe with a small quantity of vacuum grease. The use of a needle is both unnecessary and undesirable.
2. Using the syringe, apply a small bead of grease around the underside of the chamber. Evenly distribute the grease by gently pressing a spare coverslip onto the bottom of the chamber.
3. Remove and discard the coverslip. Clean away any grease which may have entered the bath area. Pay particular attention to the perfusion input and outlet ports since the presence of grease in these areas will impede the flow of perfusate.

Brush technique

The brush technique is performed in exactly the same manner as described above except that the vacuum grease is applied using a #1 or #2 artist's dotting brush. Brushes can be found in your local art shop, university bookstore, or can be purchased from Warner.

NOTE: We suggest the brush technique since the resultant application of vacuum grease is more precise.

Installing the culture dish insert

1. Begin by applying a thin coating of vacuum grease to the flat bottom of the culture dish insert.
2. Perfusing solution is delivered and removed through PE-160 polyethylene tubing which is attached to the inlet and exit ports. Make attachments as described below and run a small amount of perfusate through both the inlet and exit ports. This will minimize the introduction of bubbles after the insert is placed in position.
3. Assuming that your culture dish contains cells and media, place the assembled culture dish insert on top of the media. Secure the insert into place by gently pressing it onto the culture dish bottom.

PERFUSION

Perfusate is delivered to the chamber through 1/16" OD polyethylene tubing (PE-160, available from Warner Instruments). A tubing sample is inserted into the chamber during shipping to identify the input port.

Insertion of perfusion tubing into the input port can be greatly simplified by cutting the PE-160 on a bias rather than with a square face. We recommend pre-filling tubing with buffer before insertion as this will reduce the occurrence of bubbles in the flow path.

Solution removal is via the metal aspirator attached to the outboard end of the chamber. Solution depth can be set by adjusting the height of the aspirator.

Fluid control

The selection of solution sources and rate of delivery can be of either manual or automatic design and is left to the user. However, Warner Instruments manufactures several perfusion control systems (such as the valve-driven **VC-8** and **VC-8M Control Systems**), all of which can be used with this application.

Control of the solution delivery rate can be of a pumped or gravity feed design. A reference by Trese Leinders-Zufall describing the advantages of different perfusion control systems is available for download from the Support section of our website (http://www.warneronline.com). To facilitate fine control over the flow rate, Warner Instruments offers a dedicated flow regulator (**FR-50 Flow Regulator**) details of which which can also be found on our website.

Multiple perfusion solutions

Warner Instruments multi-port manifolds (**MM or ML Series**) can be used to connect up to 8 solution lines to the **RC-37FS** chamber. Connect the manifold output tube to the input port of the chamber. Cut tubing ends on an angle before insertion and push in as far as they will go. Air should be removed from each feed line by pre-filling with its appropriate solution. We recommend making the connection between the manifold and chamber as short as possible to minimize solution exchange times.

Suction/Level control

Removal of solution from the **RC-37WS** and **RC-37FS** is performed by aspiration. We recommend the use of a vacuum trap to avoid introduction of aspirant into your house vacuum lines. A suction tube is mounted to an adjustable holder. Adjust the vacuum pressure and solution height until the suction rate is equal to the flow rate into the chamber.

MAINTENANCE

Cleaning of the **RC-37WS** and **RC-37FS** should be performed using a dilute detergent solution. Alternatively, Warner instruments has developed a trisodium phosphate (TSP) wash protocol which is effective in cleaning plastic parts. Contact our Technical Support staff or download the protocol in PDF format from our website. (http://www.warneronline.com)

NOTE: Do not use alcohol, ether or other solvents on plastic parts.