

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 design of the **RC-37 Series** Culture Dish Inserts incorporates a diamond-shaped bath which has been shown to produce a laminar flow across the chamber. Since bath volumes are generally small, exchange times are measured in seconds even when flow rates are less than 1 ml/min.

THE RC-37WC AND RC-37FC

The **RC-37WC** and **RC-37FC** Culture Dish Inserts are small volume imaging chambers featuring rapid solution exchange, short working distances and a closed bath. The inserts 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-37WC** is designed for use with Willco (D35522P and D3522B) and Corning (25000) 35 mm culture dishes. The **RC-37FC** is designed for use with BD/Falcon (35-4077) 35 mm culture dishes.



The closed design of the chambers promotes an even and continuous solution exchange with no alteration in focus due to changes in bath height. Studies using gassed solutions can also be performed with no change in partial pressure at the chamber interface. A round coverslip seals the top of the bath resulting in a distance of 1 mm between the top coverslip and culture dish base. The chamber features a hold down ring to secure the top coverslip into place.

ASSEMBLY

A general procedure for the assembly of the **RC-37WC** and **RC-37FC** Culture Dish Inserts is to first attach a coverslip to the chamber top, then connect the flow lines, and finally to fix the assembly into the appropriate culture dish. The culture dish can then be mounted to your microscope in the usual manner.

The **RC-37WC** and **RC-37FC** are supplied with appropriately sized #1 coverslips (0.13-0.17 mm thickness), a tool (pry bar) for removing the coverslip retainer ring from the chamber top, and 3 m of PE-160 tubing. The **RC-37WC** uses 15 mm round coverslips while the **RC-37FC** uses 18 mm round coverslips.

Application of vacuum grease

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

Syringe technique

1. Begin by loading a 1cc syringe with a small quantity of vacuum grease. The use of a needle is unnecessary and undesirable.

2. Begin by removing the coverslip retainer from the top of the chamber using the pry bar. Remove the installed coverslip. Place aside for later use.
3. Using the 1 cc syringe, apply a small bead of grease around the seat to which the top coverslip rests. Evenly distribute the grease around the seat by placing a spare coverslip into the chamber top and gently press it into position.
4. 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.
5. Place a clean coverslip into the greased chamber top. The final seal is achieved by installing the retainer ring which is pressed into place. Be aware that the retainer should be oriented such that the small indentation around the outside of the retainer is towards the top. (This indentation facilitates removal of the retainer.)

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.

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 surgical.

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 through PE-160 polyethylene tubing which is attached to the inlet and exit ports. Make attachments as described in the section labeled Perfusion 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. Attach a syringe to the output flow line before placing the insert into the culture dish.
4. Assuming that your culture dish contains cells and media, gently place the assembled culture dish insert on top of the media. Using the syringe, remove the trapped air bubble from underneath the sealed insert top.
5. Once the trapped air pocket has been removed, secure the culture dish insert into place by pressing it onto the culture dish bottom.

NOTE: The RC-37 Series Insert can be secured into place by the use of vacuum grease or by the supplied retaining clips on the DH-35i or QE-1.

PERFUSION

Perfusate is delivered to the chamber through the supplied PE-160 tubing. Insertion of perfusion tubing to the input port can be greatly simplified by cutting the end of the tube on a bias rather than with a square face. We recommend pre-filling all perfusion lines before connection to reduce the occurrence of bubbles in the flow path.

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.

The rate of solution delivery can be of a pumped or gravity feed design. While these approaches allow control of the flow rate, Warner Instruments also offers a dedicated solution flow regulator (Model **FR-50 Flow Regulator**). 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>).

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-37** chamber. The manifold output tube is attached to the input port of the chamber. We recommend making the connection between the manifold and chamber as short as possible to minimize solution exchange times. Air should be removed from each feed line by pre-filling with its appropriate solution.

MAINTENANCE

Cleaning of the RC-37 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.