WARNER INSTRUMENT CORPORATION

Series 20 Chamber Instructions

SERIES 20 CHAMBERS

A common feature of the Series 20 chambers is the use of a glass coverslip as the bottom or floor of the chamber. The coverslip is applied to the chamber bottom and the assembly is then placed in the appropriate platform. The platform clamps the assembly together providing a seal between the chamber and coverslip. The assembled chamber is then mounted on a microscope stage directly or with an adaptor plate.

Coverslip Sizes: The coverslips supplied with the chamber are No. 1 (thickness range 0.13mm - 0.17mm) which can be used with all microscope systems including confocal. Other thicknesses may be used with these chambers as well. A small supply of coverslips are supplied with each chamber. Additional No. 1 coverslips may be ordered from us or from one of the suppliers listed in the appendix 5,6. The following is a list of the chambers and coverslips used:

Model	Bottom Coverslip	Top Coverslip	Model	Bottom Coverslip	Top Coverslip
RC-21	22 x 40 mm	12 mm dia.	RC-24	22 x 40 mm**	none
RC-21A	22 x 40 mm	15 mm dia.	RC-24E	22 x 40 mm**	none
RC-21B	22 x 22 mm	22 x 22 mm	RC-25	12 mm dia.	none
RC-21BR	25 mm dia.	25 mm dia.	RC-25F	15 mm dia.	none
RC-22	22 x 40 mm	none	RC-26	22 x 40 mm	none
RC-22C	22 x 40 mm	none	RC-26G	22 x 40 mm	none
RC-23	22 x 40 mm	22 x 40 mm*	RC-26GLP	22 x 40 mm	none
RC-23D	22 x 40 mm	22 x 40 mm*	RC-26DRIP	22 x 50 mm	none

* Top coverslip supplied is lexan (plastic) and may be drilled for electrode access.
** A 22 x 22 mm size may also be used with this model.

CHAMBER ASSEMBLY

Coverslips are attached to the chamber bottom with silicone vacuum grease (Dow Corning)1. Most models are machined with a 1mm wide x 0.8mm deep sealant goove in the area where the coverslip attaches. Other models have a milled relief for the round coverslip (Models RC-25 and RC-25F). Load a small (1cc) syringe with vacuum grease (Dow Corning or other stopcock grease) and carefully apply an even bead around the entire groove. If the syringe tip is too large, heat the tip and pull it to a smaller size. Place a sample coverslip (not the one to be used) on the chamber bottom and press it against the chamber to evenly distribute the grease. Remove the coverslip and carefully clean away any excess grease, especially in the bath area or the connecting perfusion channels. This is important to insure proper solution flow. Place the coverslip to be used in the experiment on the chamber being sure to align it so that the glass covers the entire groove. Set the assembly in the appropriate platform and secure it in place with the two clamps. Tighten the screws evenly to obtain a seal.

Platform/Chamber compatibility is shown below. Platforms with the "H" in the number are heater versions.

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Platform/Chamber compatibility is shown below. Platforms with the "H" in the number are heater versions.

PLATFORM	USE WITH CHAMBER MODEL
P1 or PH1	RC-21, 21A, 22, 22C, 23, 23D, 24, 24E, 26, 26G, 26GLP & 26 DRIP
P2 or PH2	RC-21B &21BR
P3 or PH3	RC-25
P4 or PH4	RC-25F

ASSEMBLY OF ENCLOSED TYPE CHAMBERS - RC-21, RC-21A , RC-21B & RC-21BR

These cell culture chambers use a second coverslip for the chamber top. Special assembly instructions are included with these models.

MOUNTING ON THE MICROSCOPE

The assembled chamber/platform can be mounted directly to the microscope stage if the stage is flat and the hole or cutout in the stage is smaller than the platform. In most cases, the stage cutout is larger than the platform and an adapter is required. The platform was designed to fit the microscope slide 76 x 26 mounting frame (p/n 471719) on the Zeiss Axiovert inverted microscope. No adapter is needed for this mounting. Stage adapters are available for the popular microscopes as listed below. Custom adapters can also be provided.

Microscope Mfg./ Model No.	**Stage Adapter**
Nikon Diaphot	SA-NIK
Nikon TMS	SA-NIK/TMS
Olympus IMT	SA-OLY
OLYMPUS IMT-2	SA-OLY/2

PERFUSION

The perfusing solution is delivered through 1/16" OD polyethylene tubing (Becton Dickinson PE-160)2. A sample of the tubing is inserted in the chamber when you receive it to demonstrate how it attaches to the chamber. Remove the sample and attach the delivery tubing to the perfusion input. Cutting the tubing on a bias rather than square will make it easier to attach. It is usually best to fill the tubing with solution before attaching to the chamber. Fluid control can be manual or automatic and is left to the user. Sources for electrically controlled valves or automated systems are listed in the appendix 2.

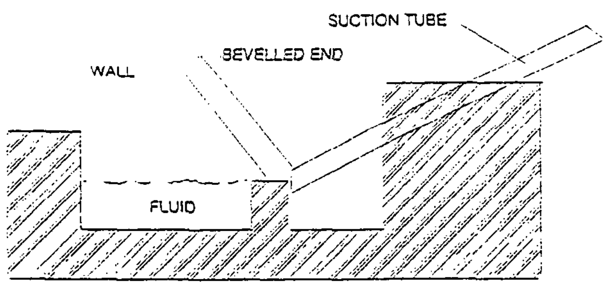
MULTIPLE PERFUSION SOLUTIONS

The multi-port manifolds MP-2, 3, 4, 5, 6, 7 & 8 are used to connect up to 8 solutions to the Series 20 chambers. Input and output tubings are PE-160 tubing. Attach the input tubings to the MP manifold. Cut the tubing ends on an angle before inserting and push the tubing in as far as it will go. Run each solution through its line to remove air. Then connect the single manifold output tubing to the chamber. Make the connection between the manifold out and chamber in as short as possible so that solution changes will be fast.

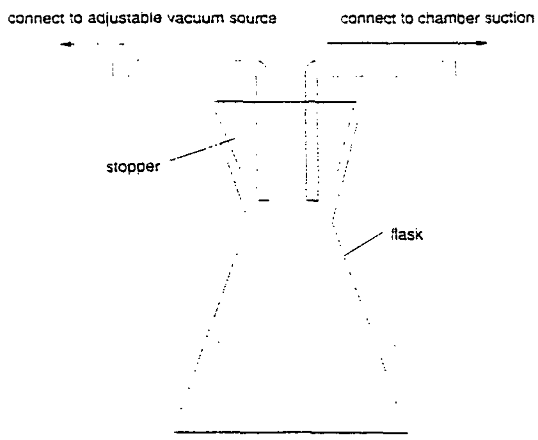
SUCTION

Removal of the solution is done by suction. The suction tubing is installed in either a hole or slot in the chamber. This permits adjustment of the fluid level in the open style chambers. However, for quiet operation, the suction tube should be positioned against the dividing wall in the suction reservoir as shown. The enclosed chambers use the same PE-160 tubing for suction and can be used with the open chambers if desired. The metal tube supplied with the open chambers has been found to work well and accepts 1/16" ID tubing.

Adjustment of the vacuum line is important to minimize noise in the chamber. The vacuum should be throttled down until the suction is just able to keep up with the flow into the chamber. A typical setup for the suction line is shown below.



Suction tube placement



A typical suction trap

HEATING WITH HEATER PLATFORMS PH-1, PH-2, PH-3 & PH-4

Heat is transferred to the aluminum platform from the pair of 20Ω power resistors, one mounted on each side. The power source should be a well filtered variable dc current supply to avoid the introduction of noise to the chamber. Connection is made with the pair of connector assemblies supplied. The heaters can be connected in series or parallel, depending on the voltage of the supply (see below for maximum parameters). Current should be limited to 1 ampere for series connection and 2 amperes for parallel.. In practice, currents less than 0.5 amperes are sufficient to maintain temperatures in the ranges normally required (35 to 40°C).

Automatic heat control can be acheived by using a heater controller such as the **Warner TC-324 or TC-344** (dual channel) to provide the heating power. The desired temperature is set and automatically maintained to less than 1°C deviation.

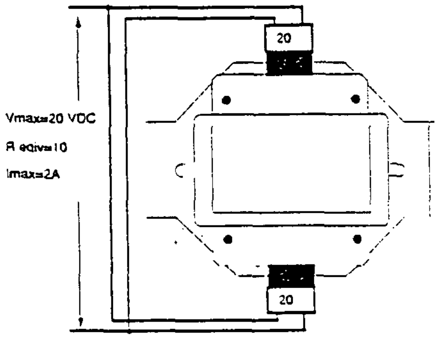


Fig. 1A PARALLEL CONNECTION

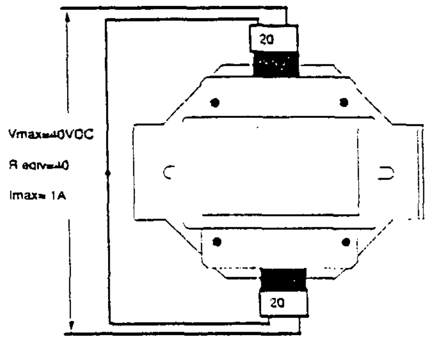


Fig. 1B SERIES CONNECTION

MONITORING THE HEAT

Heater platforms are supplied with a small thermistor assembly (see below for specifications and temperature curve characteristics). The temperature of the platform is monitored by measuring the resistance of the attached thermistor. Adjust temperature by raising or lowering the voltage to the heaters. A second thermistor or other temperature sensing device should be placed in the bath to monitor and control the bath temperature.

To control bath temperature while perfusing, a more elaborate temperature control system will be required3.

THERMISTOR INFORMATION

The thermistor supplied with the heated platform is a Thermometrics MA100GG103C (probe type) with a resistance of 10 kΩ at 25°C. Maximum temperature rating is 60C°. The thermistor is inserted in the hole drilled in the side of the platform. **NOTE: If the thermistor fits loosely in the hole, use a drop of immersion or mineral oil to insure good thermal transfer.**
Temperature vs. resistance data is shown below for the temperature range of interest.

Temp. in °C vs. Resistance in Ohms

Temp.	Resistance	Temp.	Resistance	Temp.	Resistance
16	14997.7	28	8777.79	39	5546.53
17	14321.6	29	8408.68	40	5327.34
18	13679.8	30	8057.31	41	5117.97
19	13070.4	31	7722.43	42	4917.94
20	12491.6	32	7403.29	43	4726.77
21	11941.6	33	7098.42	44	4543.91
22	11418.9	34	6808.36	45	4369.33
23	10922.0	35	6531.31	46	4200.84
24	10449.5	36	6265.75	47	4040.81
25	10000.0	37	6016.47	48	3889.51
26	9572.32	38	5776.05	49	3743.17
27	9165.29			50	3603.10

SPECIAL FEATURE CHAMBERS

TISSUE SLICE MODELS RC-22 & RC-22C

Four clamping pins are supplied with the chamber for holding slices in place. The pins are made from 26 ga stainless steel hypodermic tubing and fit into holes in the chamber. The working end is beveled to a point. They are easily removed if any or all are not needed. The pins are slighly bent prior to insertion in the chamber to give them a snug fit. Position the pins by sliding and turning to the desired location. If the pins become loose in this procedure, remove them and introduce a slight bend in the area of the pin that lies inside the supporting hole. A needle nose plier works well for distorting the tubing.

CONTROLLED ENVIRONMENT MODELS RC-23 & RC-23D

These chambers have ports for the entry and removal of gasses in addition to the perfusion ports. The top of the chamber is sealed with a 22 x 40 mm coverslip held in place with two spring clips. A plastic (lexan) coverslip is also supplied for users requiring access to the bath. Holes are easily drilled in the plastic for the introduction of probes and electrodes.

DRIP CHAMBER MODEL RC-26DRIP

Solution reservoirs connected directly to a chamber can act as antennas and introduce electromagnetic interference (noise) into recordings. By dripping the solution, the connection between the chamber and the reservoirs is broken. The RC-26DRIP chamber utilizes a drip input design.

Dripping the solution can cause problems in maintaining a steady even flow. This effect can be minimized by placing an absorbent paper (tissue) in the drip reservoir. Once the paper is saturated, it should then act as a buffer and provide a smoother solution flow.

RECORDING REFERENCE

On open-style chambers used for recording, the reference electrode may be placed directly into the perfusion outflow reservoir or into the additional well supplied for using an agar bridge. The agar bridge is constructed by the user. If a chlorided silver wire is used for the reference, it can be placed into the small hole in the side of the agar bridge well.

MAINTENANCE

Cleaning of the polycarbonate chamber should be done with a dilute detergent solution. Do not use alcohol, ether or solvents on any plastic parts. Solvents may be used on the anodized surfaces of the platforms.

CHAMBER SUPPLIES/SPARE PARTS

We stock the following supplies for your convenience. For large quantities of coverslips or tubing, or sources for other products, see below.

Order No.	Description	Qty./Pkg.	Price/Pkg.
No.1 Thickness	**Coverslips**		
CS-12R	12mm diameter (for RC-25)	100	$ 11.00
CS-15R	15mm diameter (for RC-25F)	100	$ 11.00
CS-22S	22mm x 22mm square (for RC-218)	100	$ 8.00
CS-25R	25mm diameter (for RC-21R)	100	$15.00
CS-22/40	22mm x 40mm rectangle (for RC-21, 21A, 22, 22C, 23, 23D, 24, 24E, 26 & 26G)	50	$ 9.00
Polyethylene Tubing			
PE-160/10	.062"OD x .045"ID (1.57mm x 1.14mm) tubing	10 ft. (3.3 M)	$17.00
Replacement/Spare Parts for Heater Platforms			
HC20-1	Heater Wire & Connector	2	$ 4.00
TS-60P	Probe Thermistor	1	$32.00
Multi-Perfusion Zero Dead Space Manifolds			
MP-2	Manifold, 2 input, 1 output	1	$60.00
MP-3	" 3 input, 1 output	1	$60.00
MP-4	" 4 input, 1 output	1	$60.00
MP-5	" 5 input, 1 output	1	$70.00
MP-6	" 6 input, 1 output	1	$70.00
MP-7	" 7 input, 1 output	1	$80.00
MP-8	" 8 input, 1 output	1	$80.00

SUGGESTED SOURCES FOR SUPPLIES AND EQUIPMENT

1) Dow Corning Corp., Midland Michigan, Tel: 800-248-2481
Silicone vacuum grease (also called stopcock grease) is available from most laboratory supply companies.

2)Perfusion Valve Control Systems. Basic systems or complete systems with pinch or teflon valves (Warner Instrument VC-6)

3) Best temperature regulation is achieved by preheating the perfusion solution with an in-line heater (Warner SH-27A) in addition to warming the chamber.

4) Laboratory Disposable Products, Inc., Halendon, NJ. Tel: 201-423-4177
13mm diameter "Nunc" Thermanox (plastic) coverslips, 0.2mm thickness.

Technique for setting up the perfusion of a recording chamber for physiological studies with cultured and acutely dissociated cells

Trese Leinders-Zufall
Department of Anatomy and Neurobiology and the Program in Neuroscience
University of Maryland School of Medicine, Baltimore, Maryland

In many electrophysiological and dynamic imaging experiments, controlling the speed and height of the perfusion solution in the recording chamber is often not achieved in a satisfactory manner. A method (Fig. 1) is described here for controlling the flow of the recording solutions and therefore the precise height of the solution in most recording chambers of Warner Instrument Corporation. This report is an extract of a chapter titled 'Regulating the perfusion speed and height of microscope-mounted chambers' which will be published in 'Electrophysiological Methods for the Study of the Mammalian Nervous System' (Kocsis JD (ed), Appleton & Lange).

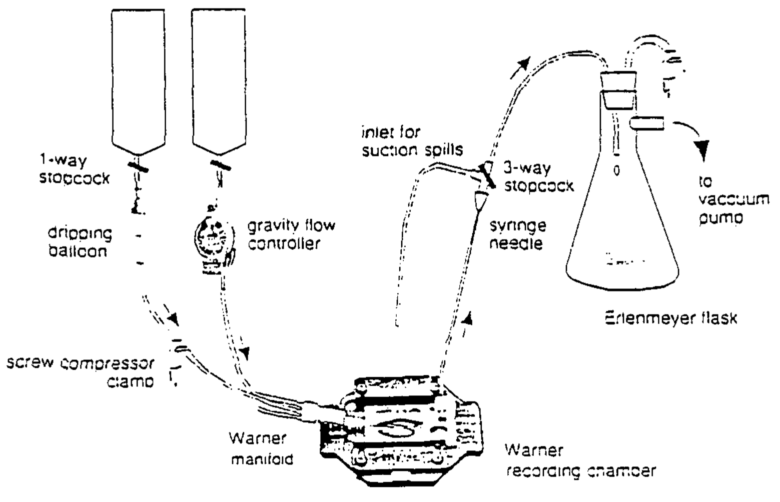


Figure 1. Schematic representation of a recording chamber set up for physiological studies with dissociated cells and/or slices.

Control of the recording chamber input

The flow of the perfusion solution into the recording chamber can be controlled by using either a pump or by gravity feed. In many laboratories it seems that gravity is one of the most commonly applied methods. However, in using this method, the speed of the solution has to be strictly controlled. There are various ways to achieve that. Some use various tubing length and diameter combined with securing the bottle or syringe containing the bulk of the perfusion solution on a specific height . This is the cheapest way, but not the most precise one. Other methods use clamping devices such as screw compressor clamps (Fig. 2 a) or tubing clamps (Fig. 2 b) to control the flow rate. Often this method is used in combination with a dripping balloon (Fig. 2 c) to visually observe the speed of the perfusate. Instead of using the various clamping devices, a more accurate method involves the use of gravity flow controllers (Fig. 2 d) that are originally designed to control the flow of intravenous solutions for hospital patients.

An often overlooked factor for the precise control of the solution flow into the recording chamber is the container that holds the bulk of the perfusion solution. When using a syringe, 'separatory funnel with a stopcock' or any other device with gravity pressure, the speed of the solution will vary proportionally to the amount of perfusate left in the container. This is caused by the 'weight loss' of the solution left in the container that provides pressure onto the flow controller. There are two ways to circumvent this problem. One might use e.g. a very large perfusate reservoir. The amount which is then lost from the container to the recording chamber is then relatively small in comparison to the remaining volume. However, it is common to forget to keep the amount of the perfusion solution above a specific point for maintaining a similar pressure on the tubing lines. For that reason, Nigel Cox of Yale University devised another solution which makes use of 60 cc glass syringes. These syringes are easy to clean and can even be sterilized or decontaminated when necessary. The glass syringes are equipped with a hollow glass plunger. If this plunger is cut open at the top and filled with weights (e.g. sand) and placed on top of the perfusate in the syringe, this weight will cause a constant pressure on the flow controllers.

In addition to controlling the flow rate of the solution, it is a good idea to have a 1-way stopcock directly at the source of the perfusate. This enables instantaneous stoppage of the solution flow, which is advantageous if more than one perfusate will be used during the experiment. Instead of the stopcock some investigators use roller clamps. However, these devices clamp down the tubing in such a way that they cause over time blockage of the solution flow due to the permanent dislocation of the tubing.

In the event that more than one bath perfusate is being used, Warner Instrument Corporation has manifolds available for up to 8 input lines. The inputs are joined internally to a common output in a near zero dead space configuration. These manifolds should be used with a very short length tubing between the manifold and the recording chamber, so the solution exchange times are minimized. In combination with the manifolds, the already mentioned 1-way stopcocks (see Fig. 1) should always be present to prevent the back-propagation of the flowing perfusion solution into the tubing of the other perfusion solutions.

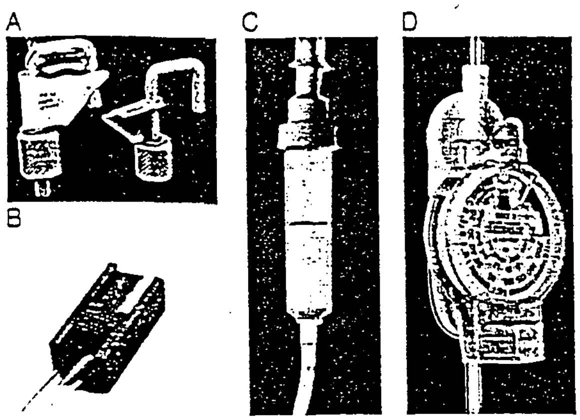


Figure 2. Various devices that help control the solution flow. (A) Screw compressor clamp (e.g. from Cole-Parmer Instrument Company). (B) tubing clamps (e.g. from Cole-Parmer Instrument Company. or Henry Schein). (C) dripping balloon for counting the amount of drops / ml that flow of the perfusate ('I.V. set', e.g. from Henry Schein or The Master Medical Corporation). (D) gravity flow controller (The Master Medical Corporation).

Control of the recording chamber output

The perfusion solution in the recording chamber can be removed by using vacuum suction. in the event that not a pump is operating the in- and output. When using vacuum suction. it is necessary to control the force of the suction precisely to be able to fine tune its height in the recording chamber. This can be achieved by using the appropriate tubing, a stopcock and the metal suction device delivered with the recording chamber.

The metal suction device is specially designed to draw air and fluid at the same time, which helps to control the solution height more accurately and also reduces electrical noise caused by the suction vortex. The ideal position of this metal suction device is such that the longitudinal opening is kept in a 90 degrees angle to the solution surface. The suction device can be easily placed at any location in the recording chamber reservoir with the use of modeling clay. in the event that the designated holder built into the chamber is at an inconvenient position.

Polyethylene tubing (PE160, 1.14 mm ID. 1.57 mm OD) is then connected to the metal suction device by using tubing that fits snugly both the PE160 tubing and the suction device. This PE160 tubing ends onto a stopcock. So. that when stopping the solution flow at the input level. the suction can also be shut down to prevent drying out the recording chamber.. An easy way to connect the stopcock to the PE160 tubing is to use a 18G1 syringe needle which has the sharp tip removed. In principle only a 1-way stopcock is needed. but it is convenient to use a 3-way stopcock. The extra inlet can then be used for suctioning spills or for quick cleaning of the recording chamber.

The other side of the 1-way or 3-way stopcock will be connected to an Erlenmeyer flask that is used for collecting the waste solution (see Fig. 1). The tubing size on this side is irrelevant and usually a larger size than the PE160. The Erlenmeyer flask has two inlet openings to regulate the force of the vacuum suction. The second inlet is connected to tubing fitted with a screw compressor clamp. By changing the diameter of the tubing. the suction force is changed on the outlet line of the recording chamber. The force of the suction is now adjusted to the inlet flow speed. The fluid level in the recording chamber can then be easily adjusted by raising or lowering the metal suction device in the recording chamber reservoir.

Acknowledgment: I thank Drs. Adam Puche. Shan Chen and Roland Bock for comments on this document.

Addresses for the various material and devices:

Cole-Parmer Instrument Company
625 East Bunker Court
Vernon Hills. Illinois 60061-1844 — phone: 1-800-323-4340

Master Medical Corporation
6991 East Camelback. Bldg. B-111
Scottsdale. Arizona 85251 — phone: 1-800-962-8573

Henry Schein
5 Harbor Park Drive
Port Washington. NY 11050 — phone: 1-800-872-4346

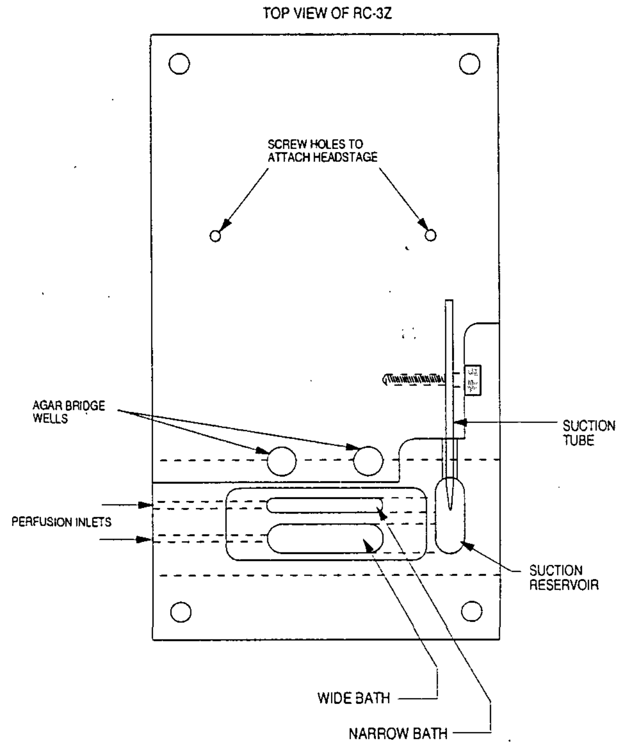
Warner Instrument Corporation
1125 Dixwell Avenue
Hamden. CT 06514 — phone: 1-800-599-4209

WARNER INSTRUMENT CORPORATION

INSTRUCTIONS FOR OOCYTE CHAMBER RC-3Z

Model RC-3Z is a simple low cost chamber for oocyte studies. It features two slot shaped bath wells; a narrow well for applications requiring rapid perfusion and a wider well which permits better electrode access. The perfusion inputs accept standard 1/16" OD (PE-160) polyethylene tubing. Solutions are removed from the suction well via an adjustable suction tube. Solution height is adjustable by raising or lowering the suction tube.

Mounting holes are present for attachment of the Warner oocyte clamp headstage . Two isolated wells are provided for making agar bridge connections to the bath.



Specifications

Material:	Polycarbonate
Footprint (L x W):	13 cm x 7.7 cm
Bath Dimensions	
Narrow Bath:	27 mm L x 3.2 mm W x 3.2 mm H
Approx. Volume:	85 µl/mm height
Wide Bath:	27 mm L x 6.3 mm W x 3.2 mm H
Approx. Volume:	170 µl/mm height
Chamber Floor Insert:	25 mm x 75 mm x 1 mm polycarbonate
Input Tubing:	1/16" OD (PE-160)
Output Tubing	1/16" ID

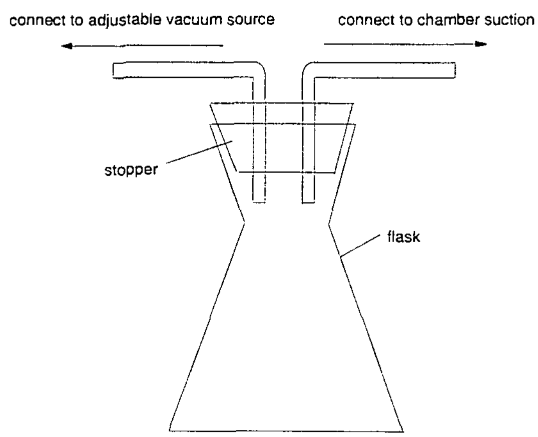
Preparation

The chamber is supplied with a 25 x 75 mm polycarbonate slide for the chamber floor. The slide is attached at the factory with RTV (734) cement. The RTV remains pliable and allows for removal of the slide if a leak occurs or if the slide needs replacement. A standard 25 x 75 x 1 mm glass slide can be substituted for better optical clarity if desired. To replace the slide, insert a thin blade between the slide and chamber taking care not to dig or gouge the chamber bottom. Remove any remaining residue, apply a thin coating of RTV to the bottom and place the new slide in position. Gently press the slide into place to distribute the cement and allow to dry. RTV sets up in approximately 30 minutes and completely cures in 24 hours.

Using the Chamber

Connect the perfusion input tubing to the desired bath well. To prevent solution from flowing from the unused bath input, insert a short length of tubing into the input hole and either pinch it off or position it so that the outboard end is higher than the bath input.

Connect a length of 1/16" ID tubing to the suction tube and connect the outboard end to a vacuum source (see sketch for suggested method). Turn on the perfusion and adjust the vacuum to a level that removes the perfusate without inducing tidal flucuations in the bath. This adjustment can be difficult. Too strong a vacuum can suck the bath dry or cause fluctuations in the bath height. A properly adjusted vacuum will keep up with the input flow with the suction tube end partially submerged so that it is simultaneously drawing both solution and air. Once the suction is properly adjusted, the solution height in the bath can be set by moving the suction tube end up or down.



Suggested vacuum connection for chamber suction.

Chamber Grounding

A ground reference electrode or chlorided silver wire can be placed either directly in the bath or in the adjacent suction well. Two wells are furnished for grounding with agar bridges. Refer to the instrument manual for suggestions on proper electrode placement if a Warner OC-725 bath clamp headstage is employed.

Maintenance

The chamber may be cleaned with ordinary laboratory detergents. It may also be sterilized by autoclaving.

DO NOT USE ALCOHOL TO CLEAN THE CHAMBER.

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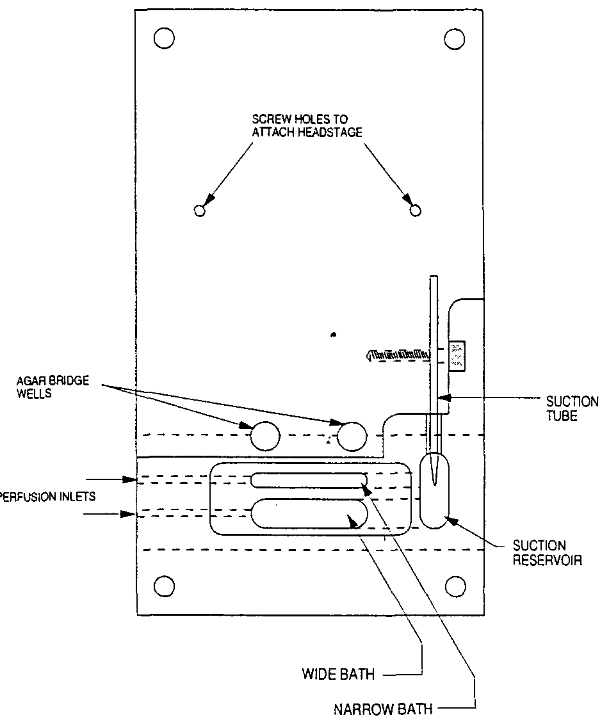

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Output Tubing	1/16" ID

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1141 Dixwell Avenue • Hamden, CT 06514 USA • Tel: 203 776 0664 • 800 599 4203 • Fax: 203 776 1278
email: sales@warneronline.com • web site: www.warneronline.com

Using the Chamber

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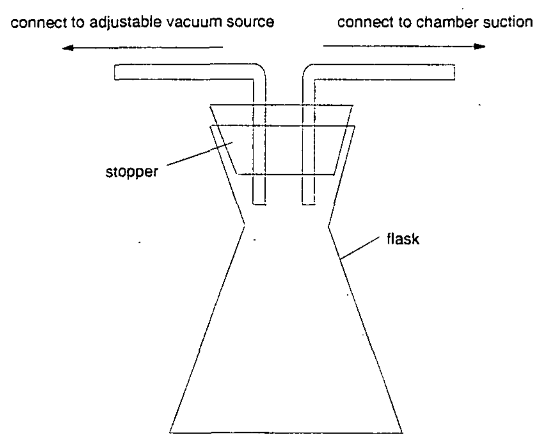

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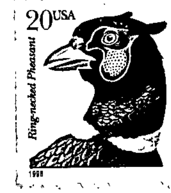
Maintenance

The chamber may be cleaned with ordinary laboratory detergents. It may also be sterilized by autoclaving.

DO NOT USE ALCOHOL TO CLEAN THE CHAMBER.

WARNER
INSTRUMENT
CORPORATION

1125 Dixwell Avenue
Hamden, CT 06514



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WARRANTY REGISTRATION CARD

2/27/01

Dear Customer:
Thank you for your purchase. It is important that you register your name as a user so that we may inform you of any changes or modifications concerning this model. We will also keep you updated on new products as they become available.

INSTRUMENT: Model OC-725C S/N: 1574

Name ________________ Title ________

Institution/Company ________________

Department ________________ Phone ________

Address ________________

City ________________ State ______ Zip ________

Country ________ Fax ________

WARRANTY REGISTRATION CARD

2/27/01

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INSTRUMENT: Model OC-725C S/N: 1573

Name ________________ Title ________

Institution/Company ________________

Department ________________ Phone ________

Address ________________

City ________________ State ______ Zip ________

Country ________ Fax ________

WARRANTY REGISTRATION CARD

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INSTRUMENT: Model OC-725C S/N: 1572

Name ________________ Title ________

Institution/Company ________________

Department ________________ Phone ________

Address ________________

City ________________ State ______ Zip ________

Country ________ Fax ________