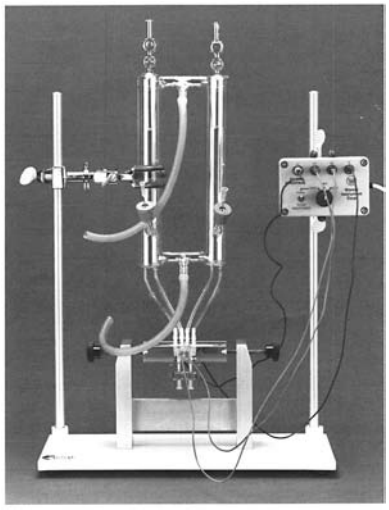
Warner Instruments, Inc.
Classic Ussing Chamber Systems
Models U-9500 and U-9500CU



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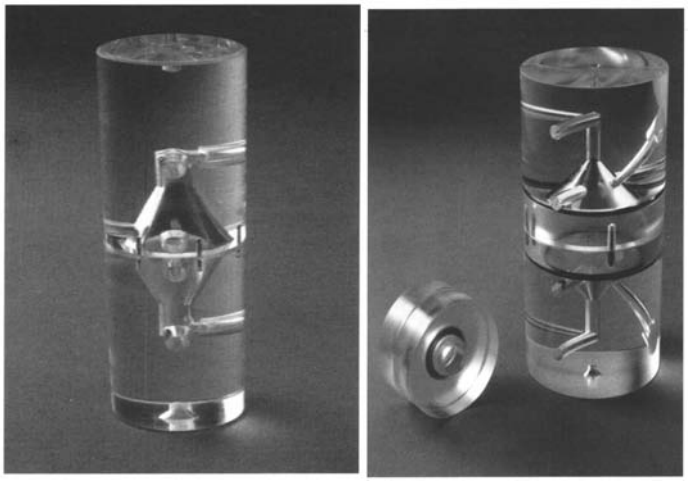
The “classic” Ussing chamber systems from Warner Instruments varies little from the original equipment developed by Dr. Ussing in the 1940’s and this tried-and-true system is still the most widely used equipment in the study of electrophysiological properties of epithelial tissues, particularly the investigation of potential difference (PD), resistance (R), and short-circuit current (Isc).

The **U-9500** and **U-9500CU** systems are complete and include chamber, support stand, circulation reservoir, ring stand, 3-prong clamp, air/gas fitting kit and electrode set. Ussing chambers are available in a variety of sizes and shapes, and the choice of chamber depends on the species and epithelia under study. In addition, **CU** style chambers are designed to accommodate culture inserts. Chambers are machined from solid clear acrylic into two symmetric halves with locating holes on the outside end for mounting onto the support bracket. One face has sharp stainless-steel pins which mate with holes in the corresponding face for puncturing and positioning the epithelium membrane. Luer-tapered fittings are installed into ports for making electrical I/O connections into the chamber.



Classic and CL style (culture insert) Ussing chambers

The circulation reservoir is hand-blown from borosilicate glass and is available in four fluid capacities. Two glass condensers are included to minimize evaporative losses. Reservoir openings are provided to allow the introduction of compounds at any time during the experiment.

Standard Accessories For The U-9500 Series

The following accessories are included with the **U-9500**:

- Electrode sets; two Ag/AgCl (voltage) and two Ag wire (current)
- Electrode bridge fittings (6 voltage, 6 current)
- Circulation reservoir
- Support stand and 3-prong utility clamp

- 3/16" ID tubing for circulation reservoir connections (10”)

NOMENCLATURE

Text conventions

To minimize the potential for confusion, we have employed several text conventions which are specified below. Since our goal is to provide clarity rather than complexity, we welcome any feedback you may wish to provide.

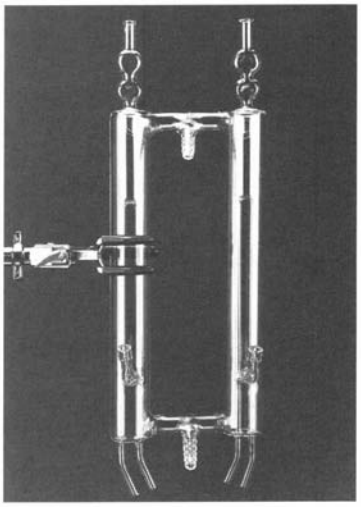
- Warner Instrument product numbers are presented using **bold type**.
- References to system components are specified using UNDERLINED SMALL CAPS
- References to sections on system components are specified using NON-UNDERLINED SMALL CAPS
- Special comments and warnings are presented in highlighted text.

Any other formatting should be apparent from context.

THIS EQUIPMENT IS NOT DESIGNED NOR INTENDED
FOR USE ON HUMAN SUBJECTS

ASSEMBLY OF THE U-9500

The initial assembly of the **U-9500** Ussing chamber system is straightforward. The apparatus is cleaned and pre-assembled without the chamber in place. Once the experiment is ready to proceed, the chamber is assembled and the assembly completed.


Circulation reservoir

1. Begin by attaching the supplied gold RING STAND RODS to the RING STAND BASE.
2. Mount the glass CIRCULATION RESERVOIR onto one of the vertical RING STAND RODS using the 3-PRONG CLAMP. The second RING STAND ROD can be used as an additional support for the RESERVOIR, or optionally for mounting the headstage of an amplifier such as the Warner instruments EC-800.
3. Place the two EVAPORATION CONDENSERS into the top of the CIRCULATION RESERVOIR.

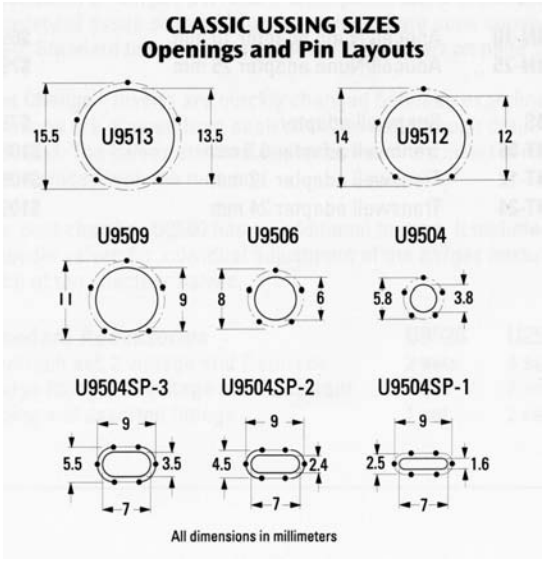
Preparations For An Experiment

1. Chamber half-cells and cartridge inserts should be cleaned prior to use. Chambers are best cleaned using bleach and distilled water. (*See* Appendix for instructions.) Uncouple any tubing which connects the CHAMBER to the RESERVOIR. Disassemble the CHAMBER by unscrewing the CLAMP SCREWS and remove the two chamber halves.
2. All peripheral items; equipment, components, and media must be prepared before assembling the chamber and clamping the two halves together. This includes preparation of agar bridges, the air/gas mixture, application of water sources, and mounting of tissue samples. Descriptions are provided below.
    - Prepare electrode agar bridges. Procedures for bridge preparation can be found within the Appendix. Each half cell requires separate bridges for voltage and current passing electrodes.
    - The air/gas mixture (usually 95% 0_2/5% CO_2) should be readied for connection from the regulator to the chamber.
    - The water source (i.e. circulation bath, pump) should be readied for connection to the RESERVOIR WATER JACKETS. Water should be maintained at the desired temperature (normally 37°C).
    - Verify that the buffer/electrolyte to be used in the experiment is prepared (normally a Krebs/Ringer solution). RESERVOIRS and CHAMBERS come in different sizes and can

require as much as 35 ml for initial filling. Additional volume will be required to replace fluid lost through normal evaporation, as well as any fluid removed for sampling or buffer exchange during the course of the experiment.

Assembly of the CHAMBER

The Ussing chamber consists of two half-cells manufactured from clear acrylic and consists of an aperture surrounded by narrow, sharpened PINS on one side, and mating holes for these pins on the other side. As shown to the right, chambers come with either three or five mounting PINS, depending on the chamber size.



Tissue slices should be excised and mounted to the chamber half before assembly. If using cell culture inserts, these should be mounted in their respective adapters or cartridges (mounting instructions are included with each adapter).

Mount the tissue to be studied into the sharpened PINS on one chamber half and carefully close the two halves together. If using a CULTURE INSERT (Model **U-9500CU**), place the INSERT into the chamber half before closing the two halves together. Chamber halves contain GUIDE PINS to facilitate orientation during assembly.

After assembly of the CHAMBER, the four pre-assembled white PERFUSION FITTINGS should be in alignment with each other. These fittings contain a luer-taper at the base for installation in the chamber ports and a barbed top for connections to tubing. When properly mounted to the SUPPORT STAND, these four fittings will be along the CHAMBER top.

Final Assembly

The assembled CHAMBER is mounted onto the SUPPORT STAND by centering the SCREW CLAMPS with the two guide holes located on the round surface of each chamber half and tightening each SCREW CLAMP until the chamber is secured in the approximate center of the stand. When the CHAMBER is properly mounted, the four DOWN-TUBES of the CIRCULATION RESERVOIR will be in approximate alignment with the four PERFUSION FITTINGS of the CHAMBER. Allow approximately two inches of clearance between the FITTINGS and the DOWN-TUBES for tubing adjustment. Connection between the FITTINGS and DOWN-TUBES will allow circulation of buffer from the CIRCULATION RESERVOIR into the CHAMBER and back again.

The assembled CHAMBER contains ports for voltage and current electrodes and ports for perfusion tubing. The VOLTAGE ELECTRODE PORTS are machined at a 45° angle to the axis of

the chamber and are positioned 90° from the CHAMBER top. When the chamber is mounted in the SUPPORT STAND these ports will be along the front. The remaining two ports are the CURRENT PASSING ELECTRODE PORTS and will be along the back of the CHAMBER.

Once mounted in the SUPPORT STAND, connections to the CHAMBER should be made in the following order:

1. Insert the VOLTAGE ELECTRODES into the front PORTS. Push gently until they are seated against the internal stops.
2. Insert the CURRENT PASSING ELECTRODES into the rear PORTS. Push gently until they are seated against the internal stops.
3. Connect the DOWN-TUBES of the RESERVOIR to the PERFUSION FITTINGS of the CHAMBER. 3/16" ID tubing is ideal for connecting to the glass DOWN-TUBES and 1/8" ID tubing is best for connecting to the PERFUSION FITTINGS. This disparity in size is easily remedied by using 3/16" ID tubing to connect to the DOWN-TUBES, and using 1/8" ID, 3/16" OD tubing to connect to the PERFUSION FITTINGS. The OD of the tubing attached to the PERFUSION FITTINGS will then neatly slide into the ID of the tubing attached to the DOWN-TUBES.
4. Attach the *Gas-Lift* air lines. The two ports located on each side of the glass CIRCULATION RESERVOIR are termed SEPTUMS. These are provided for connection to your air/gas line and are used to both oxygenate the buffer and energize the *Gas-Lift* mechanism of buffer circulation. Warner Instruments includes a GAS LINE CONNECTION KIT with the **U-9500**, but other means of connection are certainly achievable. To use the included kit, simply slip one rubber stopper over each SEPTUM so it is snugly secured. Plunge the supplied 23 gauge hypodermic needle into the back of the rubber stopper, and connect the plastic part of the needle to 1/4" ID tubing. The other end of this tubing is then connected to the air/gas manifold or regulator system of your lab. **Do not begin air/gas flow yet**.
5. Attach the temperature controlled water lines to the WATER JACKET. The remaining two ports on the CIRCULATION RESERVOIR (positioned on the upper and lower CROSS ARMS) are provided for connection into the RESERVOIR WATER JACKET. Connect these ports to your temperature controlled water source (usually a circulation bath) using 1/4" ID tubing. One connection supplies water IN and the other provides for water OUT (it makes no difference which is which).

OPERATION

1. Load the RESERVOIR and CHAMBER by pouring buffer from a beaker into the well on the RESERVOIR top (remove the EVAPORATION CONDENSERS). Depending on the porosity and flow-through characteristics of the tissues or monolayer studied, buffer may or may not

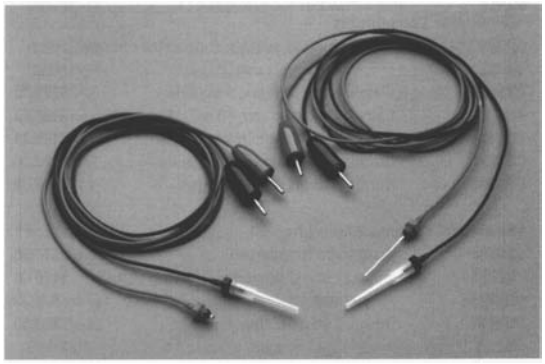
flow freely from one half-cell into its mating half-cell. If not, simply fill each half-cell separately. Replace the EVAPORATION CONDENSERS once the RESERVOIR is filled.

2. Begin air/gas flow from the regulator. Slowly apply pressure until the desired bubbling action is achieved in each RESERVOIR half. The typical half-cell requires a pressure of 0.5 PSI or less to maintain the *Gas-Lift* action for the duration of an experiment. Once the half-cells are bubbling, the *Gas-Lift* action will circulate the buffer throughout each half-cell as well as provide buffer oxygenation.

   NOTE: Open the valves **slowly** and use care to maintain only a minimal flow to each half-cell as excess velocity (and pressure) can render the tissues or monolayers useless for experimentation.

3. The apparatus is now ready for electrical connections. If you are using electrode bridge fittings and Ag/AgCl electrodes, the 2 mm pins of the electrodes can be connected directly to the headstage of the voltage clamp. If you are using agar bridges and tubing, the tubing must first be connected to an electrolyte holding vessel (i.e. a beaker), and then connected to the voltage clamp via a low resistance connector (usually calomel half cells). The instructions included with your voltage clamp will dictate the procedure to be followed.

PREPARATION OF ELECTRODES AND BRIDGE FITTINGS


Electrode and bridge fittings

The electrode bridge fittings (model **U-9565EP**) are modified micropipette tips with a Luer-taper that matches the respective insertion ports of the **U-9500** chamber. The barrel end of the fitting is female threaded to accept male-threaded Ag/AgCl (voltage) electrodes and Ag wire (current) electrodes (model **U-9975A**). The longer fittings are used with the voltage electrodes which must be placed close to the membrane surface. The tips are cut at a 45° angle to expose the largest possible tip area to the membrane. Electrodes are color-coded; red for positive and black for negative. Additionally, the voltage electrodes are Ag/AgCl and the current passing electrodes are 1 mm silver wire.

Assembling the electrode bridge fittings with wire electrodes.

The male thread of the electrode (current or voltage) is screwed clockwise into the female thread of the agar filled bridge fitting. Assemble Ag/AgCl voltage electrodes with the longer bridge fittings and Ag wire electrodes with the shorter fittings. Once the electrode/bridge fittings are assembled and filled with agar (*see* Appendix), they can be mounted into the

chamber. NOTE: Ag wire must be chlorided prior to assembling the current electrodes. (*see* Appendix)

APPENDIX

Chamber cleaning

Cleaning the chambers is best performed using bleach and distilled water. Do not use alcohol to clean the **U-9500**, as it can cause cracking, fracturing, etching or melting of the plastic parts. **Do not autoclave.** In the rare circumstances where sterilization is necessary, use gas (thylene oxide) or gamma radiation techniques. Also, do not remove any of the permanently mounted, threaded components from the chambers as this can damage the threads or cause the plastic to crack.

Storing electrodes

After completion of an experiment the fittings and electrodes must be stored and/or cleaned.

If radioisotopes or other contaminating materials have not been used during the course of the current experiment, the entire electrode/fitting assembly can be stored with the agar bridge intact and reused in future experiments. Simply submerse the assembly in a container filled with the buffer media (Ringer/KCl) and store at room temperature. This procedure can be repeated until the agar dries out or until the initial voltage offset drifts beyond tolerable limits.

If you are not planning an experiment in the near future, or if radioisotopes have been used, or if the agar has dried out, the electrodes and fittings should be disassembled and cleaned. The fittings should be cleaned with DDH_2O water only. Use a long, thin object (e.g., pipe cleaner, thin gauge needle, etc.) to remove all agar media from the inside of the fittings. The electrodes can be cleaned with distilled water, saline, or ETOH and a soft cloth or brush. **Do not use acetone or strong solvents to clean. Do not autoclave.**

If the pellet surface of the voltage electrodes are especially dirty or corroded, a new surface can be exposed by gently abrading with a fine grit (600-1000) sandpaper and then cleaning. **Do not use Emory cloth to abrade the surface.** After cleaning, store the electrodes in a clean, dry amber plastic or glass container.

Chloriding silver wires

Before using Ag wires as current electrodes, they must be chlorided. New (previously unused) wire should first be cleaned with ETOH before continuing, while previously chlorided wire should have the old chloride coating removed. (Use the same technique as described in the preceding section.) As with a new wire, remove finger oils by cleaning with ETOH before proceeding.

Two methods are commonly used to chloride Ag wire; soaking a clean wire in household bleach or electroplating a clean wire using a voltage source. Both methods are described below.

A) Soaking in bleach - Simply immerse the wire in full strength common household bleach (Clorox) for 15 to 30 minutes until a purple-gray color is observed. Rinse and use.

B) Electroplating - Electroplating a silver wire with chloride is achieved by making the wire positive with respect to a solution containing NaCl (0.9%) or KCl (3M) and passing a current through the electrode at a rate of 1 mA/cm^2 of surface area for 10-15 seconds or until adequately plated (a 1 cm length of 1 mm diameter wire will require approximately 0.3 mA). The color of a well plated wire should be purple-gray. Periodic reversal of the polarity while plating the electrode tends to yield a more stable electrode.

   When electroplating a previously plated wire, you may find that it does not plate evenly. Complete removal of the residual silver chloride is usually necessary to effect a uniform coat. Before making the wire positive to the chloriding solution, reverse the polarity for 5 to 10 seconds to remove any remaining chloride that might be left in pits on the wire. Then proceed as described above.

Agar bridges

If you are using prepared agar bridge tubing rather than Ag/AgCl electrodes for the electrical connections from the Ussing chamber to the voltage clamp, the bridge fittings can still be used. Supplied with the chamber are connectors that are male threaded at one end (for insertion into the bridge fitting) and barbed at the other end for insertion into 3/32" ID tubing. Once this connection is completed, the entire assembly can be formed as one continuous agar bridge.

Filling bridge fittings with agar

1. Prepare an agar bridge solution of 2-5% agar with 3M KCl or a Ringer solution containing KCl. Heat this mixture to boiling and allow to cool to 65-75° C.
2. Fill a syringe with a large gauge hypodermic needle (18 gauge or larger) with the hot agar/Ringer solution.
3. Slowly inject the agar/Ringer solution into the tip of each bridge fitting, making sure that the tip and fitting is completely filled and that no air bubbles form. Attach the electrodes to the fittings allow the solution to cool and solidify within the fittings. The agar solution will properly encapsulate the Ag/AgCl pellet or Ag wire during solidification.
4. After solidification, the electrode bridge fittings are ready to insert into the Ussing chamber. The fittings should slide easily into their respective ports using a slight

forward push and clockwise twisting motion, until they bottom-out. Do not use excessive force as this can cause the fittings to break.

5. After all electrode bridge fittings are properly mounted in the chamber, connect the 2 mm pins of the electrodes to their respective jacks of the voltage clamp headstage and proceed with the Ussing chamber/voltage clamp experiment.

Available tissue and culture cup cartridge inserts

Tissue and culture cup inserts are available from Warner Instruments for use with the **U-9500** and **U-9500CU** Ussing chamber systems.

Tissue adaptors

Catalog Number	Description
U-9524A-04	3.8 mm round with o-ring
U-9524A-06	6.0 mm round with o-ring
U-9524A-09	9.0 mm round with o-ring
U-9524A-12	12.0 mm round with o-ring
U-9524A-13	13.5 mm round with o-ring
U-9524B-04	3.8 mm round with 3 pins
U-9524B-06	6.0 mm round with 5 pins
U-9524B-09	9.0 mm round with 5 pins
U-9524B-12	12.0 mm round with 5 pins
U-9524B-13	13.5 mm round with 5 pins
U-9524C-03	1.6 x 7 mm slotted with 6 pins
U-9524C-04	2.4 x 7 mm slotted with 6 pins
U-9524C-05	3.5 x 7 mm slotted with 6 pins

Culture cup inserts

Catalog Number	Description
U-9524F-09	Falcon adapter – 9 mm
U-9524F-12	Falcon adapter – 12 mm
U-9524F-25	Falcon adapter – 25 mm
U-9524M-12	Millicell adapter – 12 mm
U-9524M-30	Millicell adapter – 30 mm
U-9524N-10	Anocell/Nunc adapter – 10 mm
U-9524N-25	Anocell/Nunc adapter – 25 mm
U-9524S	Snapwell adapter

U-9524T-06	Transwell adapter – 6.5 mm
U-9524T-12	Transwell adapter – 12 mm
U-9524T-24	Transwell adapter – 24 mm

Reorder parts

Chambers

Catalog Number	Description
U-9504	Classic Ussing chamber, 3.8 mm diameter
U-9506	Classic Ussing chamber, 6.0 mm diameter
U-9509	Classic Ussing chamber, 9.0 mm diameter
U-9512	Classic Ussing chamber, 12.0 mm diameter
U-9513	Classic Ussing chamber, 13.5 mm diameter
U-9504SP-1	Slotted Ussing chamber, 1.5x7.0 mm
U-9504SP-2	Slotted Ussing chamber, 2.5x7.0 mm
U-9504SP-3	Slotted Ussing chamber, 3.5x7.0 mm
U-9521	Cell culture Ussing chamber

I/O electrode components

U-9565EP	Electrode bridge fittings (20)
U-9565A-16	Agar bridge connectors (1/16” ID, set of 10)
U-9565A-18	Agar bridge connectors (1/18” ID, set of 10)
U-9565T	Agar bridge tubing (1/16” ID x 100 ft, PE)
U-9975A	Electrode Set, Voltage (Ag/AgCl pellet), Current (silver wire), 2 ea.

Circulation components

U-9304	Circulation reservoir, 4 ml/side
U-9310	Circulation reservoir, 10 ml/side
U-9320	Circulation reservoir, 20 ml/side
U-9330	Circulation reservoir, 30 ml/side

U-9302	Condenser (set of 2) all sizes

Additional components

U-9303	Air/gas connection kit
U-9403	Support stand (includes one support stand rid)
U-9403R	Support stand rod
U-9404	3-prong utility clamp