Warner Instruments, Inc.
Single Channel Ussing Chamber
Model U-9926

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The model **U-9926** is a self contained single channel Ussing chamber. The unique design uses plug-in cartridges allowing for fast, convenient set-up and changing of the tissue or culture cups. A large variety of plug-in cartridges are available for the **U-9926**.

The **U-9926** is comprised of three parts; a base plate, two chamber half-cells, and an insert cartridge.

1. The chamber half-cells mount onto the base plate and are held in place with an upright member through which two chamber clamping screws are held.
2. The two chamber half-cells are secured by the clamp screws. The two mirror-image halves contain the solution bath reservoirs, gas and electrode ports, and the warming solution water jackets. When assembled. the top of the chamber is slotted to accept the cartridge insert.
3. The cartridge insert is a two piece male-female assembly for holding the tissue or culture cup. After the tissue is mounted or the culture cup installed, the assembled cartridge is inserted into the chamber and the chamber clamps tightened.

STANDARD ACCESSORIES FOR THE U-9926

The following accessories are included with the **U-9926**:

- Electrode sets; two Ag/AgCl (voltage) and two Ag wire (current)
- Electrode bridge fittings (5 voltage, 5 current)
- Air/Gas Luer connectors (6 ea.)
- 1/16" ID "Y" connectors (2 ea.)
- Drain valves (2 ea.)
- 3/16" ID tubing for water jacket connections (10')
- 1/16" ID tubing for air/gas connections (6')

NOTE: The Luer connectors used with this chamber are modified versions of standard Luer types. The modification is accomplished by cutting 3 mm from the small end of a standard Luer fitting.

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**.
- 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

CONNECTIONS TO THE U-9926

Electrodes, air/gas lines, warming solution lines, and drain plugs are connected to the chamber through ports located on the chamber half-cells (*see* Figure 1, below).

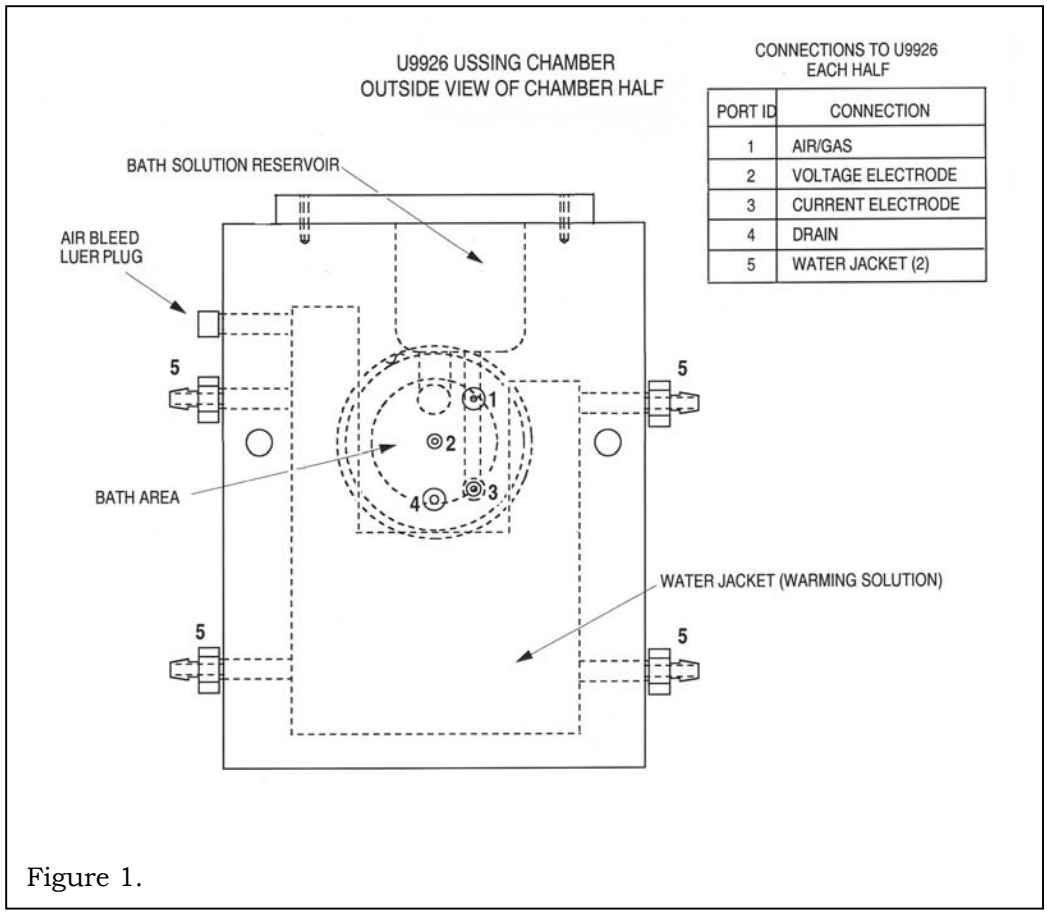

Figure 1.

Ports 1 and 4 are Luer taper holes which accept standard male Luer fittings that have been shortened in length by 3 mm. Any standard plastic male Luer fitting can be made acceptable by removing 3 mm from the length.

Port 4 functions as a convenient drain when changing solutions or replacing the cartridge. Continuous perfusion can also be effected by connecting this port to a pump and running the pump output to the chamber top.

PREPARATIONS FOR AN EXPERIMENT

1. The chamber half-cells and cartridge inserts should be cleaned prior to use. Disassemble the chamber by unscrewing the clamp screws and remove the two chamber halves. Uncouple any tubing connecting one half to the other. Chambers are best cleaned using bleach and distilled water. (*See* Appendix for cleaning instructions.)

2. All peripheral items; equipment, components, and media, must be prepared *before* mounting the cartridge insert into the chamber and clamping the two halves together. This includes preparation of agar bridges, the air/gas mixture, application of the water sources, and mounting of the chamber inserts. Descriptions are provided below.

    - Electrode agar bridges must be prepared. Procedures for their preparation can be found within the Appendix. Each half cell requires separate bridges for the voltage and current electrodes.

    - The air/gas mixture (usually 95% 0_2 / 5% CO_2) should be readied for connection from the regulator to the chamber. Air/gas, supplied from your regulator, is connect to port 1 on *each* chamber half (*see* Figure 1) and connections are made using the modified Luer connectors supplied with the **U-9926**. A tubing set with connectors (including a "Y" connector) is supplied.

    - Using the supplied 1/16" ID tubing, make a connection from the air/gas source to the "Y" connector. Do not make connections from the “Y” connector to the chamber halves yet.

    - The water source (i.e. circulation bath, pump) should be readied for connection to the integral water jackets of the chamber. Flexible tubing with an ID of 0.187 inch will properly fit the barbed inlet and outlet port fittings. The water should be maintained at the desired temperature (normally 37° C).

    - Verify that the buffer/electrolyte to be used in the experiment (normally a Krebs/Ringer solution) is prepared. The assembled chamber will require approximately 18 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.

    - If using cell culture inserts, these should now be mounted in their respective adapters or cartridges (mounting instructions are included with each adapter). If using tissue slices for the experiment, these should now be excised and mounted to the pin-style inserts.

OPERATION AND EXPERIMENTAL PROCEDURES

1. Assemble the two half-cells on the chamber base using the two clamp screws (*see* Figure 2, below). Allow sufficient clearance for insertion of the cartridge.

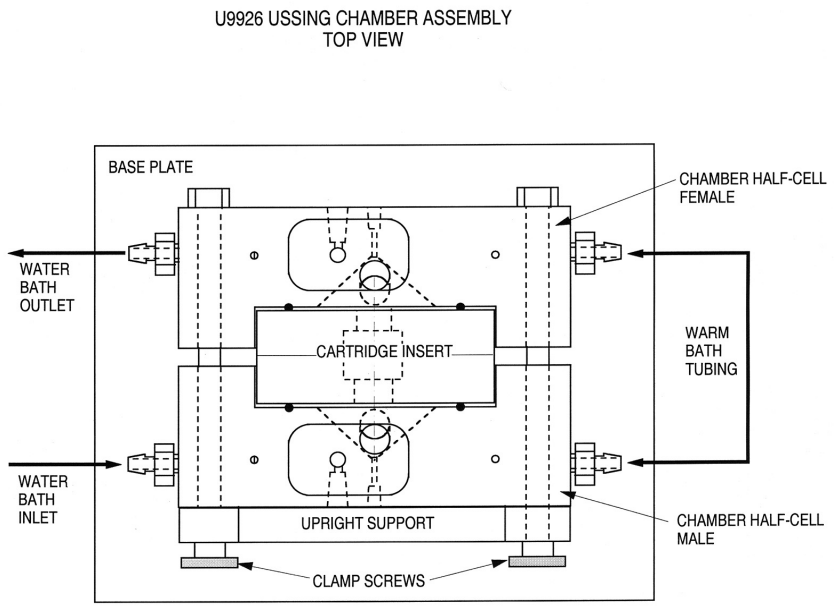

Figure 2

2. Place the cartridge inserts into the loosely assembled chamber halves (note that the large hole is on the bottom and will align with the center of the chamber when inserted). After inserting the cartridges, securely tighten the screw clamps but do not over-tighten.

   NOTE: The clamps must be equally tightened for the O-rings to seat properly on the sides of the cartridge inserts. If misaligned or improperly tightened, the chamber will leak. Visually observe the size of the gaps on each side of the chamber halves and adjust the clamp screws to make this gap spacing equal.

3. The warming solution can now be connected in series to the chamber half-cells as follows: Connect the water circulation source with 3/16" ID tubing to the water inlet port (marked port 5 in Figure 1). A second Luer port is directly above water inlet port to aid in the removal of trapped air from the system. Using a short length (6") of tubing, connect the output port (5) to the input port of the other half cell. Complete the circulation loop with a length of tubing from the remaining port to the water source. Begin water circulation to bring the chamber to the desired operating temperature. Trapped air can easily be removed

by loosening the Luer plugs in the chamber halves. Be sure to firmly reseat the plugs once the air has been expelled.

4. Connect the air/gas source (from the "Y" connector") to the air/gas inlet ports on each side of the assembled chamber (port 1 in Figure 1). **Do not begin air/gas flow yet**.

5. Insert all electrode bridge fittings into their respective ports. Push gently until they are seated against the internal stops.

6. Insert the 2-way stopcock valves into the drain ports. These valves are tapered and should be pushed forward until they seat snugly. Close the valves by turning the handles to a position that is perpendicular to the valve body. During the course of the experiment, these ports can be used to dispense sample fluids from the chambers or for buffer exchange by simply opening the valves until the desired volume is dispensed. Be sure to replace the removed fluid by pouring fresh buffer into the top reservoirs. After completion of the experiment, these ports provide a convenient way of draining the buffer from the chambers before separating the two halves.

7. Load the chambers by pouring buffer from a beaker into the reservoirs located on the chamber top. Depending on the porosity and flow-through characteristics of the tissues or monolayers studied, the buffer may or may not flow freely from one half-cell into its mating half-cell. If this is the case, simply fill each half-cell separately. Put the reservoir covers in place AFTER the reservoirs are filled.

8. Begin the air/gas flow from the regulator. Slowly apply pressure until the desired bubbling action is achieved in each reservoir. 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.

9. 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

The electrode bridge fittings (model **U-9565EP**) are modified micropipette tips with a Luer-taper that matches the insertion ports (ports 2 and 3 in Figure 1) of the **U-9926** 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 voltage sensing and black for current passing.

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, they can be mounted into the chamber.

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

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.

APPENDIX

Chamber cleaning

Cleaning the chambers is best performed using bleach and distilled water. Do not use alcohol to clean the **U-9926**, 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. A simple method to remove the chloride coating from silver wire is to quickly pass the wire through a flame. A properly flamed wire will be bright silver in color. 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.

Available tissue and culture cup cartridge inserts

Tissue and culture cup inserts are available from Warner Instruments for use with the **U-9926**, **U-2500** and **U-5000** Ussing chamber systems.

Tissue inserts

Catalog Number	Description
U-9924A-04	3.8 mm round with o-ring
U-9924A-06	6.0 mm round with o-ring
U-9924A-09	9.0 mm round with o-ring
U-9924A-12	12.0 mm round with o-ring
U-9924A-13	13.5 mm round with o-ring
U-9924B-04	3.8 mm round with 3 pins
U-9924B-06	6.0 mm round with 5 pins
U-9924B-09	9.0 mm round with 5 pins
U-9924B-12	12.0 mm round with 5 pins
U-9924B-13	13.5 mm round with 5 pins
U-9924C-03	1.6 x 7 mm slotted with 8 pins
U-9924C-04	2.4 x 7 mm slotted with 8 pins
U-9924C-05	3.5 x 7 mm slotted with 8 pins

Culture cup inserts

Catalog Number	Description
U-9924F-09	Falcon adapter – 9 mm
U-9924F-12	Falcon adapter – 12 mm
U-9924F-25	Falcon adapter – 25 mm
U-9924M-12	Millicell adapter – 12 mm
U-9924M-30	Millicell adapter – 30 mm
U-9924N-10	Anocell/Nunc adapter – 10 mm
U-9924N-25	Anocell/Nunc adapter – 25 mm
U-9924S	Snapwell adapter
U-9924T-06	Transwell adapter – 6.5 mm
U-9924T-12	Transwell adapter – 12 mm
U-9924T-24	Transwell adapter – 24 mm

Reorder parts

Catalog Number	Description
U-9565SC	Electrode Bridge Fittings (20) 10 ea. for Voltage Electrodes 10 ea. for Current Electrodes
U-9975A	Electrode Set (4) 2 ea. Voltage (Ag/AgCl pellet) 2 ea. Current (silver wire)
U-5565SC	2-Way Stopcock Valve (4) with modified male Luer adapter (use for drain)