

The classic style Ussing Chamber from Warner Instruments is comprised of two half-cells manufactured from clear acrylic (Lucite or Plexiglas) and consisting of an aperture surrounded by narrow, sharpened pins on one half-cell and mating holes on the other half-cell. Chambers are available with aperture diameters of 3.8, 6.0, 9.0, 1.2, and 13.5 mm and each diameter has five tissue mounting pins (except for the 3.8 mm chamber which has only three pins). The assembled chamber (two joined half-cells, henceforth referred to as the CHAMBER) also contains various ports as described below. Chamber halves contain guide pins to facilitate proper assembly.

Following assembly of the half-cells, the four pre-assembled white fittings along the top of the chamber are aligned with each other. Each fitting contains a Luer-taper at its bottom for installation into its respective CHAMBER port and has a barbed top for tubing connections. We refer to these fittings as the *TOP fittings*.

The Chamber face has two 45° angle ports which are machined 90° from the four *TOP* ports. These are the Voltage Electrode ports and are referred to as the *FRONT fittings*. The remaining two ports (unless drain ports are included) are situated 180° degrees from the two *FRONT fittings* and are where the current passing electrodes connect to the Chamber. These are referred to as the *BACK fittings*.

The assembled Ussing Chamber is mounted into its support stand by centering the screw clamps on the guide holes located on the round surface of **each Chamber (opposite each aperture)**. Tighten each clamp until the Chamber is secured in the approximate center of the support stand.

Using a 3-prong clamp, the glass circulation Reservoir is now mounted onto one of the vertical ring stands (both clamp and ring stand are included with the **U-9500 Complete System**). A second ring stand can be used as additional support for the Reservoir, or optionally for mounting the headstage of a voltage clamp amplifier. When properly mounted, the four down-tubes of the Reservoir should be in approximate alignment with the four *TOP fittings* of the Chamber. Allow approximately two inches of clearance between the *TOP fittings* and the base of the down-tubes for final adjustments.

Now connect the four down-tubes of the Reservoir to the four *TOP fittings* of the Chamber. These connections facilitate circulation of buffer from the Reservoir, into the Chamber, and back again. Connect 3/16" ID tubing to the glass down-tubes, and 1/8" ID-3/16" OD tubing to the *TOP fittings* of the Chamber. The two tube sets are coupled by inserting the 3/16" OD tubing (from the *TOP fittings*) into the 3/16" ID tubing (from the down-tubes).

The two apertures located on both the left and right side of the glass circulation Reservoir (termed Septum's) are for connection of the air/gas line (which oxygenates the buffer and facilitates gas-lift circulation of the buffer). Warner includes a connection kit with the **U-9500 Complete System**, but other means of connection are achievable. To use the included kit, simply slip the rubber stopper over the Septum so it is snugly secured. Then plunge a 25 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 tube is connected to the air/gas manifold or regulator system of your lab.

The remaining two apertures of the circulation Reservoir (upper and lower) are for connection to the water jacket for thermal control. Connection is made via 1/4" ID tubing to your source of temperature controlled water (usually a circulation bath). Make one connection *in* and the other *out*.

U-9521 Cell Culture Insert Ussing Chamber

The use of permeable cell culture inserts (porous membrane filters, culture cups, and culture wells) has become widespread in recent years. The numerous advantages of this application have revolutionized in vitro cell biology and it is now used extensively in electrophysiological studies of epithelia. The U-9521 Ussing chamber is designed to hold culture cups via specially made, low-cost adapters. This is an extremely versatile and economical system for using cell culture inserts in transepithelial studies. Warner has available adapters in a large variety of sizes for most brands of culture inserts.

The adapter is mounted in the standard "classic" Ussing support stand. Procedures and connections for experimentation are the same as described above. Instructions for assembly of the culture cup and adapter are provided with each adapter set.