MULTI-96

Resistance measurement chamber for 96-well Millipore Multiscreen permeability and PAMPA plates

INSTRUCTION MANUAL

Serial No._____________________

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Warranty

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of one year* from the date of receipt. WPI’s obligation under this warranty shall be limited to repair or replacement, at WPI’s option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI’s approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. In some instances, photographic documentation may be required. Some items are time-sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container.

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

* Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.
MULTI-96

Introduction

WPI developed the MULTI-96 as an electrode system dedicated to resistance measurement in permeability and PAMPA (parallel artificial membrane permeability assay) plates. Non-cell based 96-well permeability or PAMPA assays were specifically designed for predictive drug candidate testing. To ensure that the donor/acceptor fluxes are not due to porous or unstable hexadecane or phospholipid layers, the stability of the hexadecane or phospholipid membrane can be tested at the end of the incubation period by electrical resistance measurements, in a manner similar to TEER measurement by using EVOM or EVOMX.

WPI's EVOM and Millipore's Millicell-ERS can be used for measurements with MULTI-96 although the EVOMX, which provides a BNC recorder output and a toggle switch, is recommended.

MULTI-96 is designed to be used with the following 96-well plates from Millipore:
- MultiScreen-IP for PAMPA assays (no underdrain) # ELIIP 10 SSP
- MultiScreen-IP for PAMPA assays (with underdrain) # MAIP N4 510
- MultiScreen Permeability plate # MAPB MN 310

Electrode care

Silver/silver chloride voltage electrodes may exhibit a small voltage drift when dry and newly immersed in electrolyte solution. This drift may affect potential measurements but not resistance measurements. Leaving both cap and chamber electrodes with saline solution in the chamber for at least 20 minutes, with the cable connected to EVOM and the power off, will often stabilize the electrode. In the “off” position, EVOM connects the voltage electrodes, allowing them to equilibrate more quickly.

The current electrodes are made of pure silver. The surface, however, is chlorided before shipping for better stability and faster response time. With time, the silver chloride surface may break down and leave pure silver exposed. This should not change the performance of Endohm-Multi significantly. In any case, if the user wishes to re-chloride the surface, the procedure is very simple: pour a small amount of laundry bleach (such as Clorox) into the chamber, let it stand for 15 minutes, then rinse it out. The newly chlorided surface will look dark gray in color just as it did before breaking down. If some areas do not appear this color, the most likely cause is that the surface is coated with some sort of contamination. In this case, use a cotton swap to rub the surface of the electrode when immersed in the bleach (to remove the contamination), then repeat the chloriding procedure.
The structure of MULTI-96 is pictured below. The bottom of the chamber contains four pairs of concentric electrodes: a voltage-sensing silver/silver chloride pellet in the center and an annular current electrode. This special arrangement is designed to reduce the resistance variation between the center and corner well of the blank plate. The current electrodes are made of silver and coated with silver chloride. The cable connecting the chamber to EVOM can be easily disconnected from both the top cap assembly and the bottom chamber.

**Sterilization**

MULTI-96 may be sterilized with EtO, alcohol, or a bactericide (for example, Cidex or Sporicidin). *No organic solvents should be used.* The black and clear section of the base, made of polymethyl methacrylate (acrylic or PMMA), is the most vulnerable part for sterilization. *Any sterilization treatment that can damage PMMA should not be used.* After sterilization, the electrodes should be thoroughly rinsed with sterile perfusing solution before making membrane measurements. To maintain the sterilization, the device can be stored in the UV hood.

**DO NOT AUTOCLAVE THIS INSTRUMENT.**

**Measurement**

Add an appropriate culture media electrolyte inside the MULTI-96 chamber. Place a blank culture plate on top of the chamber. Add an appropriate amount of culture media in each well of the plate. Make sure that the height of the fluid inside the chamber is not too high so that when the top electrode is inserted, the fluid will not overflow the top of the well. For repeatable and accurate readings, it is important to let the top electrode be well seated on the chamber, ensuring that it will be centered.

For voltage measurement, the instrument should be zeroed with a “blank” cup. Adjust the “Zero V” knob on EVOM until the voltage reading is zero. This adjustment corrects for any asymmetry between the lower and upper voltage electrodes. Note that the lower voltage electrodes are connected to instrument ground. Thus, the upper electrode (inside the cup) will produce DC voltage readings relative to the bottom (ground) electrode. Readings will be opposite in sign from WPI’s STX-2 electrode. For example, a cup which produces a reading of 2 mV with STX-2 will produce a -2 mV reading with MULTI-96.

For resistance measurements, the background resistance (Rb) of the blank cup should be measured first. Rb depends on the distance between the bottom and top electrodes, the composition of the saline solution, and the temperature at which the measurement is performed. If any of these conditions change, Rb should be redetermined. *Typically, its value will be in the 150 to 250 ohm range.* That value is much higher than the regular Endohm for the single insert mostly due to the small top electrode and the very long and narrow space between the filter and the bottom of the chamber. There will also be a variation of the Rb between each well; a difference of 30 ohm between wells in the center and the wells in the corner is considered normal. That difference is due to the arrangement of the well and electrodes, and the non-uniformity of the multiwell microplate. However, all of these differences will not affect the accuracy and reproducibility of the measurement. The reproducibility of each particular well’s Rb value is better than 5 ohms. The user needs to take readings of Rb in each well and write them down. When making a resistance reading of a membrane-coated microplate, Rb of each well should be subtracted from the total resistance measured from the same well. For high resistance measurements (*e.g.*, resistance of individual hexane/hexadecane treated membrane is ~5 kohm), an Rb difference of 20 to 30 ohms is negligible. Therefore, resistance value of the membrane can be subtracted by one Rb value (for example, from the center well).