INSTRUCTION MANUAL

STX4

Electrode with Replaceable Blades
Compatible with EVOM™ Epithelial Volt/Ohm Meters

www.wpi-europe.com
082724

CONTENTS

ABOUT THIS MANUAL

The following symbols are used in this guide:

This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.

This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.

Fig. 1—The STX4 electrode is designed for use with the EVOM™ Manual meter.

INTRODUCTION

The **STX4** electrode can be used with the **EVOM™ Manual** and is designed to facilitate measurements of voltage (potential difference (PD)) and resistance (transepithleal electrical resistance (TEER)) of cultured epithelia (cellular layer) grown on permeable membranes in 12- and 24-well tissue culture plates. The STX4 can measure directly inside the well plates with 12 and 24 *inserts*. **STX4 is ideal for the 24-well** *transwell* **format**).

NOTE: These permeable membrane cell culture *inserts* are also commonly referred as *transwells*.

Each **STX4** electrode is made of two electrode blades, and each electrode blade has an outer (current) and an inner (voltage) electrode. Current is applied between two electrode blades, and resistance is measured by detecting the voltage change between the inner electrodes of the electrode blades.

The **STX4** is designed primarily for 24-well hanging *inserts* or *transwells* (for example, Corning 3470). The **STX4** can be used with 24-well high throughput screening (HTS)

plates (for example, Corning 3378) and 12-well *inserts* (for example, Corning 3460) with good results, as long as the electrode placement is consistent. Consistent electrode placement and mechanical stability are essential for consistent resistance results. Use of a stable sample temperature and the same liquid volumes will result in better reading stability and measurement consistency.

Features

- The **STX4** electrode fits ideally onto 24-well hanging *transwells* and can stay hung on top of these *transwells* (for hands-free operation). The electrode has improved measurement precision since it mitigates or minimizes variabilities contributed by electrode positioning seen with **STX2** electrode.

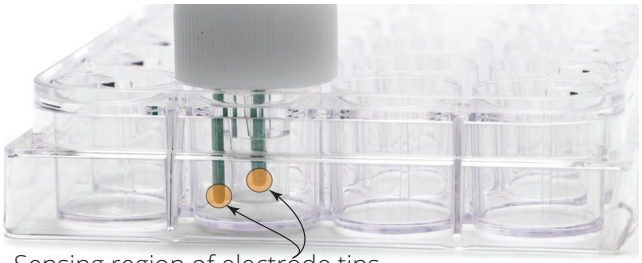

Fig. 2—The electrode tips of the STX4 electrode have a metallic color and must be immersed in the conductive liquid like a media or buffer solution.

- The electrode tip (Fig. 2) is specially coated and does not need to be chlorided with bleach or sodium hypochlorite for proper functioning, and it is not affected by chloriding either. Chloriding was critical for the previous model (**STX2-PLUS** electrodes) to maintain its functionality.
- The electrode tip (active sensing area) of the **STX4** is shorter than the **STX2-PLUS** tip. Therefore, it requires less liquid volumes than the **STX2-PLUS** to keep the electrode tips immersed and able to provide stable readouts.
- Electrode blades are replaceable. Over a period of use (for example, months or years) when the electrodes may have formed deposits from media or samples and may start showing reading instability, you may change the blades without having to replace the entire electrode.

Notes and Warnings

CAUTION: Always lift the electrode by the body, never by the cable.

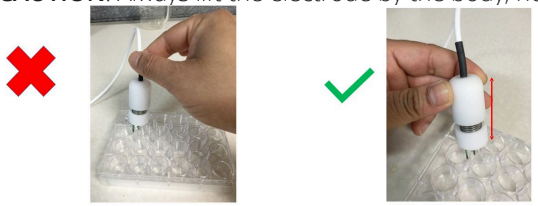

Fig. 3—(Left) Do NOT hold the electrode by the cable. It can physically break the internal connections gradually.
Fig. 4—(Right) Hold the electrode by the plastic region indicated by the red arrow.

CAUTION: Limit the liquid immersion or liquid spray level somewhere below the maximum level indicated by the arrows in Fig. 5 (Right). You don't want the liquid to get inside and reach up to the cables or connectors. You can wipe the rest of the electrode with a paper towel sprayed with isopropanol or ethanol. (Do not spray directly.)

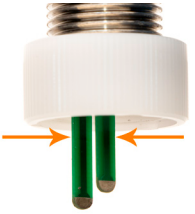

Fig. 5—Don't allow liquid to pass the line indicated by the arrows.

CAUTION: Make sure the blades of the electrode are not too deep that the blades physically touch the membrane or the bottom of the well plate. The blades should not be in direct physical contact with anything, aside from the conductive liquid (media or buffer) in which they are immersed.

Fig. 6—The inner electrode is positioned above the membrane without making contact.

CAUTION: Do NOT apply any heat or flame to any portion of the electrode or the electrode blades.

CAUTION: NEVER leave the electrode in alcohol (isopropanol or ethanol) for more than 30 minutes at a time. Continuously soaking the electrode in alcohol may weaken the protective coating on the electrode blades and can shorten the electrode's life. Electrode tips that show signs of peeling need to be replaced.

CAUTION: Do NOT use ammonia for cleaning.

CAUTION: Do NOT immerse the electrode head or any cable or connector portion in liquid. Only the electrode tip portion can be immersed in liquid.

CAUTION: Do NOT sand or abrade the electrode surfaces. The electrode blades (especially the tips) showing deep scratches need to be replaced.

Parts List

After unpacking, verify that there is no visible damage to the instrument. Verify that all items are included:
(1) **STX4** Electrode
(1) Pair of Spare Electrode Blades
(1) **99776** Wet Test Fixture
(1) Instruction Manual (available online at www.wpiinc.com/manuals)

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 27 of this manual. Please contact WPI Customer Service if any parts are missing at (941) 371-1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100 mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 27 of this manual.

OPERATING INSTRUCTIONS

Positioning Electrode in a Culture Cup

Proper placement of the electrode in the sample well is critical in making accurate measurements. The **STX4** electrode is designed to facilitate measurements of membrane voltage and resistance of cultured epithelia in tissue culture wells. The lengths of the electrodes are unequal allowing the longer (external) electrode to go inside the well of the well plate (basolateral) and the shorter one to stay on top of the *insert* (apical).

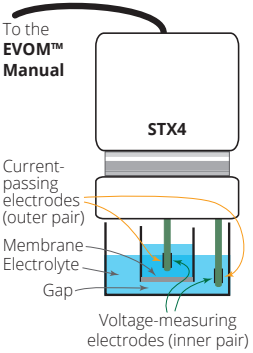

Fig. 7— (Right) Electrodes in solution.

Stable Repeatable Readings

The **STX4** is ideal for 24-well hanging *inserts* (for example, Corning 3470). The electrode's best performance can be seen in this format. The **STX4**'s electrode design allows you to set the correct depth and keep the electrode vertical and centered on top of a 24-well *transwell*. Once the electrode is placed securely, and the electrode movement ceases, a stable reading can be obtained with the **EVOM™ Manual**, negating any need to take multiple measurements. Once the electrode's working depth has been set, use the same depth across all samples to compare results among the sample set.

Ease of Use

The **STX4** electrode design allows the electrode to hang vertically (Fig. 8, left) on top of the 24-well hanging *transwells* and does not need to be handheld. Insert the outer (longer) electrode into the basal access (well of the well plate) and the shorter one on top of the membrane. Center the lower length adjustment ring onto the upper edge of the *insert* and let the electrode stand on its own. The electrode blades no longer need to be inserted at an angle (Fig. 8, center), eliminating any possibility of introducing errors associated with angle variance during measurement. The **STX4** electrode placement is stable and repeatable, as compared to the **STX2** electrode.

*For 24-well HTS plates (for example Corning 3378), keep the basal electrode of the **STX4** centered in the basal access.

*For 12-well *inserts*, the **STX4** electrode needs to be handheld.

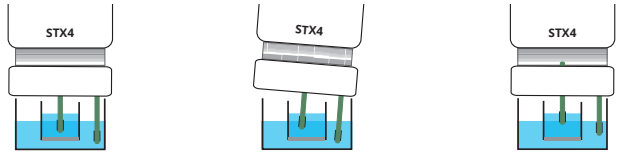

Fig. 8—The correct placement is on the left. An angle variance or depth variance could change the measurements.

Adjusting the Exposed Length of Electrode Blades

1. Firmly grasp the white adjustment ring at the end of the electrode body with one hand. With the other hand, hold the shaft of the electrode body (Fig. 9). Rotate the ring clockwise to extend the electrode blades so that they can enter deeper into a sample. Rotate it counter-clockwise to retract the electrode blades so that they can stay at a shallower depth inside a sample.

   NOTE: Ideally, the ring should at least extend a little beyond the steel surface. This allows the step in the ring to engage the outer edge of the *insert* (or the tri supports of a hanging *insert*). Adjust the ring so that the sensing (coated) region of the electrode blades (Fig. 10) stays fully immersed in liquid during measurement. A change in depth can change readings. Once set, use this length adjustment consistently to compare results in an experimental group.

Fig. 9—Rotate the front ring to adjust the exposed length of the electrode blades.

Fig. 10—The exposed electrode blade lengths are properly adjusted. The arrows highlight the coated sensing region.

2. Align the electrode blades with the well *inserts* and position the **STX4** over the well so that it rests securely in an upright position (Fig. 11, Fig. 12). (Images shown with a Corning 3470 24-well *insert* and plate.) For greater stability, rest the cable on the tabletop. For other plates and *inserts*, center the electrode blades, and the weight of the top portion of the electrode will hold the electrode in place when taking a measurement.

Fig. 11—The outer electrode reaches close to the bottom of the well plate, and the inner electrode does not touch the membrane.

Fig. 12—The STX4 sits securely over a 24-well insert.

Preparing to Take Measurements

1. Disinfect the electrode tip using 70% ethanol or isopropanol. Keep the tip immersed for 10 minutes, and then rinse it with sterile distilled water, buffer of media. (For details of the disinfection, see "Disinfecting the STX4 Electrodes" on page 9.) Wipe the rest of the blades and the electrode body with a paper towel sprayed with alcohol.
2. Position the blades of the **STX4** electrode in the well plate. (See "Positioning Electrode in a Culture Cup" on page 5.)
3. Let the electrode tips stay fully immersed in the media or buffer solution for five (5) minutes. No *inserts* or *transwells* are required. Repeat this step daily prior to use when the electrode is removed from a dried storage location.

   NOTE: The electrode may stay plugged into the EVOM™ Manual as long as the EVOM™ Manual remains powered off.
4. The electrode is now ready for taking measurements.
5. Insert the electrode into a *transwell*. The longer electrode goes onto the well of the well plate and shorter one stays on top of the membrane of the *transwell*.

CAUTION: Make sure you have enough liquid to keep the electrode tips fully immersed. For 24-well *transwell* plates (for example, Corning 3470), use a minimum of 150µL of media on top (apical) and 500µL of media on the bottom (basolateral). For 12-well *transwell* (for example, Corning 3460) use a minimum of 500µL on the top (apical) and 1000µL of media on the bottom (basolateral). You can also adjust the length of the electrode blades by rotating the adjustment ring so that the tips can stay immersed to the appropriate liquid depth.

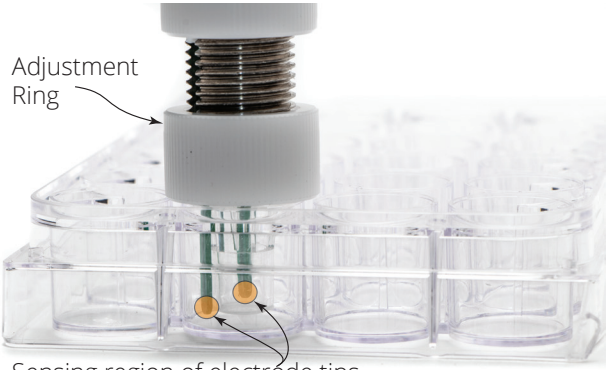

Fig. 13—Make sure the electrode tips (sensing region) is fully immersed in the media or buffer during measurement. Notice that the inner electrode does not touch the membrane and the longer outer electrode should not touch the bottom of the well plate.

6. (OPTIONAL) To minimize the chance of a sample cross-contamination, dip the electrode tips in 70% ethanol or isopropanol before measuring the next sample, and follow that with a quick dip in distilled water, buffer or a media solution.
7. After taking measurements, soak the electrode blade tips in 70% ethanol or isopropanol for 5-10 minutes, and then rinse them with distilled water and let them air dry before storage. (For details of the disinfection, see "Disinfecting the STX4 Electrodes" on page 9.)

MAINTENANCE

Electrodes must be properly cleaned, sterilized and stored.

Cleaning/Maintaining the STX4 Electrodes

1. After you finish taking measurements for the day, soak (immerse) the electrode tips in 70% ethanol or isopropanol for 5–10 minutes.
2. Rinse the electrode tips with distilled water and allow them to air dry.
3. Store the electrodes dry and in a place away from light (or with minimal light).
4. When the electrodes are used frequently, soak the electrode tips once a week in a 1% Tergazyme solution for 15 minutes. Then, rinse them well with distilled water. Enzol may be used as an alternative to Tergazyme. This step may be done just before disinfection and before beginning an experiment.

Disinfecting the STX4 Electrodes

The **STX4** electrode is resistant to most methods of low temperature (or room temperature) chemical disinfection. The electrode tips may be disinfected by immersing the tips in a disinfection solution. Wipe the rest of the electrode with a paper towel that has been sprayed with a cleaning solution. Ortho-phthaladehyde (Cidex OPA or Rapicide OPA), 70% ethanol or 70% isopropyl alcohol can be used. A solution of 5% sodium hypochlorite (undiluted household bleach) is also a good choice. After disinfecting, rinse the electrode tips in sterile water, buffer or media before using the electrode to take sample measurements.

CAUTION: NEVER leave the electrode in alcohol (isopropanol or ethanol) for more than 30 minutes at a time. Continuously soaking the electrode in alcohol may weaken the protective coating on the electrode blades and can shorten the electrode's life. Electrode tips that show signs of peeling need to be replaced.

CAUTION: Do NOT use ammonia for cleaning.

CAUTION: Do NOT immerse the electrode head or any cable or connector portion in liquid. Only the electrode tip portion can be immersed in a liquid.

CAUTION: Do NOT sand or abrade the electrode surfaces. The electrode blades (especially the tips) showing deep scratches need to be replaced.

Sterilizing the STX4 Electrodes

The **STX4** electrodes and the electrode blades are non-sterile as supplied. Acceptable low temperature sterilization methods for the electrodes include gamma irradiation and ethylene oxide gas (ETO). Chemical disinfection is generally considered adequate before using the **STX4** electrode for experiments.

CAUTION: Do NOT apply any heat or flame to any portion of the electrode or the electrode blades.

Storing the Electrodes

After a daily use, wash and store the electrodes dry away from light. Regular cleaning and maintenance is critical for proper functionality and for maintaining the functional life of the electrode blades.

CAUTION: Always lift the electrode by the body, never by the cable.

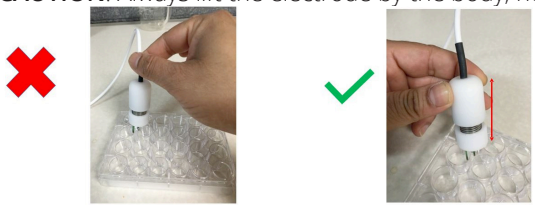

Fig. 14—(Left) Do NOT hold the electrode by the cable. It can physically break the internal connections gradually.
Fig. 15—(Right) Hold the electrode by the plastic region indicated by the red arrow.

CAUTION: Limit the liquid immersion or liquid spray level somewhere below the maximum level indicated by the arrows in Fig. 16 (Right). You don't want the liquid to get inside and reach up to the cables or connectors. You can wipe the rest of the electrode with a paper towel sprayed with isopropanol or ethanol. (Do not spray directly.)

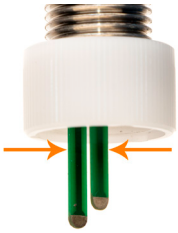

Fig. 16—Don't allow liquid to pass the line indicated by the arrows.

CAUTION: Make sure the blades of the electrode are not too deep that the blades physically touch the membrane or the bottom of the well plate. The blades should not be in direct physical contact with anything, aside from the conductive liquid (media or buffer) in which they are immersed.

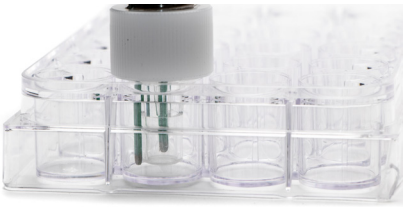

Fig. 17—The inner electrode is positioned above the membrane without making contact.

Replacing the Electrode Blades

The electrode blades do not need to be replaced unless it is required. When abnormal reading instabilities are noticed, even after a recent cleaning with Tergazyme or Enzol and 5 minutes of equilibration of the electrode tip, replacement of the electrode blades is recommended.

Follow these steps to replace the existing electrode blades with the new ones:

1. Remove the front ring by rotating the ring counter-clockwise (Fig. 18, Fig. 19).

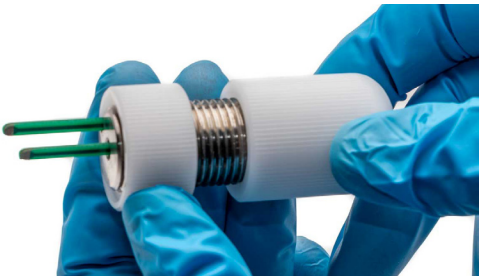

Fig. 18—Twist off the front ring to remove it.

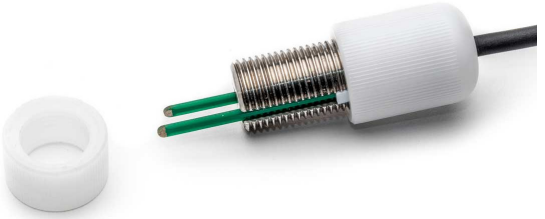

Fig. 19—Removing the front ring allows access to the blades and greater visibility for replacing the blades.

2. Remove the back of the casing by twisting it off. Rotate the casing counter-clockwise.

Fig. 20—Twist the back of the casing off to expose the connection ports for the blades.

3. Gently pull both electrode blades straight out, one at a time.

4. Identify the replacement electrode blades (Fig. 21).
   - The electrode blade with the white tab is the outside electrode.
   - The one with the positioning padding is the inside electrode.

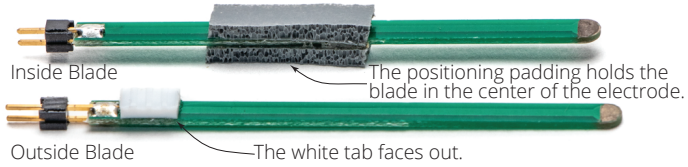

Fig. 21—The inside and outside electrode blades of STX4 electrode are different.

5. Insert the new outside electrode first. Line up the electrode in the groove with the white tab facing out (Fig. 22), align the pins with the connector and gently push the electrode blade into place until it is properly seated (Fig. 23).

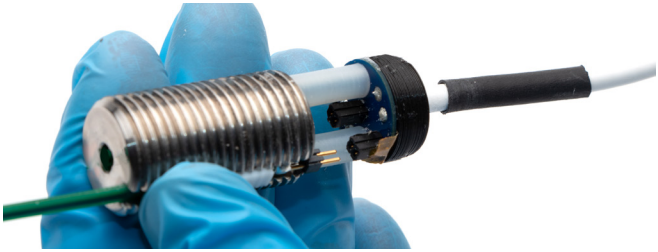

Fig. 22—Align the pins with the connection port and gently press the blade in place.

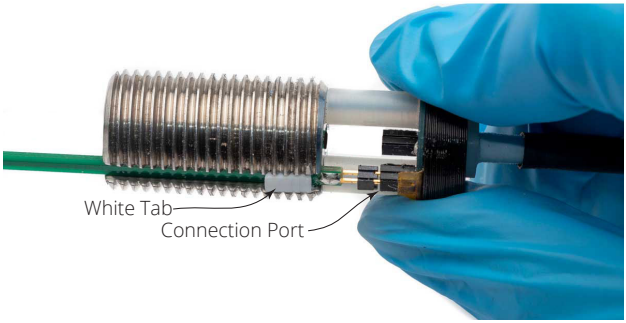

Fig. 23—The white tab faces out, the blade fits into the channel and the pins slide into the connection port. Pins should go all the way in.

5. Now, insert the new inside electrode. The inside blade needs to be inserted parallel to the outside blade. Both sides of the inside blade are identical, so it does not matter in which orientation the inside blade goes through the slot or blade's pins go into the connector. Push the blade with the foam positioning

padding through the center hole of the electrode (Fig. 24), align the pins with the connector and gently slide the pins into the connector until they are properly seated (Fig. 25). Avoid touching the coated portion of the tip.

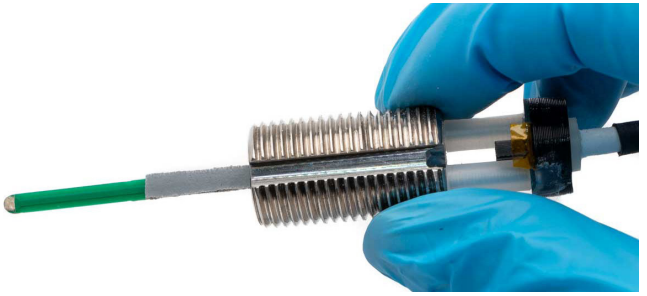

Fig. 24—Press the blade with the foam positioning padding through the center hole. The outside blade was removed so you can see the connection port.

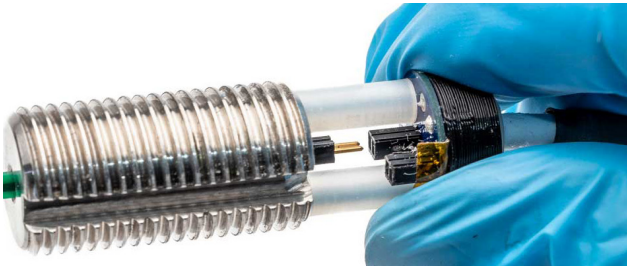

Fig. 25—Align the pins with the connection port and gently press the blade into place. The outside blade was removed so you can see the connection port.

NOTE: Erratic readings after replacement of the blades may indicate that the pins of the inner and/or outer electrode blades are not seated all the way inside the connector. A gentle pressure only is all that is needed to push the pins inside the connector.

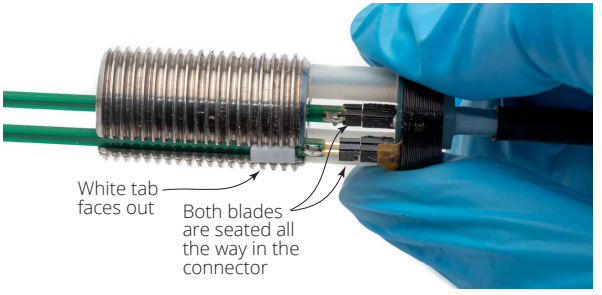

Fig. 26—Check the blade attachments to make sure the white tab of the outer blade faces out and the pins of the both blades are seated all the way inside the connector.

7. Reinstall the back of the casing by rotating it clockwise into place. Then, place the front ring of the electrode in place and rotate it clockwise to reinstall it.

Fig. 27—The STX4 electrode is assembled and ready for use.

Testing the STX4 Electrode

To test the **STX4**, use a variety of KCl (potassium chloride) solutions.

1. Prepare 40, 80 and 160 mM concentrations of KCl using deionized (DI) water. For instructions on making the KCl solutions, see “Appendix A: KCL Testing Mixtures” on page 20.
2. Add 1.5 mL of the three KCl concentrations to three different wells (in a 24-well plate) that you will use for this test. No *inserts* or *transwells* are needed.
3. Put the wet test fixture (WPI# **99776**) provided by WPI on top of a 24-well plate over the filled wells (Fig. 28, Fig. 29). The fixture ensures consistent electrode positioning. Make sure you insert the electrode into the slot so that the longer electrode stays near the wall of the wells and shorter electrode stays in the center (Fig. 30).

Fig. 28—Position the fixture over the filled wells of a 24-well plate.

Fig. 29— The test fixture is inserted all the way inside the wells of a 24-well plate.

Fig. 30—The electrode is inserted so that the long blade is near the wall of the well and the short blade is near the center of the well.

4. Measure the resistance values.

   NOTE: The 40 mM KCl solution is susceptible to reading fluctuation and may take a couple of minutes to give a stable reading.

   The electrode needs to be equilibrated for 5 minutes in a conductive liquid (for example, media, buffer or 160 mM KCl) before this test or before using the electrode for measurements. If this has not been done, let the electrode equilibrate for 5 minutes in 160 mM KCl solution before you start recording numbers. If the values are fluctuating in 80 and 160 mM KCl solutions, check the length of the electrodes. Make sure the front white ring allows the tips to stay immersed in the liquid.

   Here are the expected resistance values.

KCl concentration (mM)	40	80	160
Resistance Value (Ω)	78–130	45–75	22–38

NOTE: Resistance values are expected to be around this range. The important part is that resistance values must decrease with the increasing KCl concentration.

NOTE: These resistance readings can vary due to fluid volume and electrode depth. These values are a reference. An Excel scatter plot of the molarity vs. resistance (as a power plot) should show an R^2 value > 0.98. If the R^2 value is acceptable, then the electrode can be used even if it is slightly out of range. If the resistance readings in the KCl solutions are out of range, then the electrode may need cleaning. See "Cleaning/ Maintaining the STX4 Electrodes" on page 9. Electrodes which are not being properly maintained may begin to show instabilities indicating the need for cleaning. **Regular maintenance of the electrode is strongly recommended and needed for functional longevity of the electrode blades.**

ACCESSORIES

STX4-BLADES	Replacement Blades for **STX4**, pkg. of 3 pairs
99675	EVOM2 Adaptor Cable
99776	STX4 Wet Test Fixture
7363-4	Enzol Enzymatic Detergent
13740	Alconox Powdered Precision Cleaner
504611	Rapicide OPA/28 High Level Disinfectant

Fig. 31—Plug the STX4 into the 99675 cable to use the electrode with an EVOM2. The 99675 cable is sold separately.

TROUBLESHOOTING

Issue	Possible Cause	Solution
Electrode topples over when released	The cable is heavy and can cause the electrode to tip over if too much of the cable is in the air.	Lay the cable on the tabletop as close to the electrode head as possible.
The reading appears to be drifting* (see the drift definition below).	The electrode tips (sensing region) may have deposits of cell culture media constituents.	The electrodes tips (sensing region) require enzymatic (Tergazyme or Enzol) cleaning.
	The fluid temperature in the plate is changing.	Equilibrate the plate at room temperature.
		Use a plate warmer or hotplate.
	In a 5% CO_2 environment, a loss of CO_2 causes the media pH to change, and the resistance reading may change. This is mainly applicable in the context of continuous measurement for an extended period (hours, as compared to a few minutes).	A 5% CO_2 environment can help in reducing pH media changes. A plate warmer or hotplate can reduce outgassing.
The electrode reading is unstable or fluctuating. (See the stability definition below).	The electrode tips may not be fully immersed in adequate conductive liquid (media or buffer).	Rotate the front electrode length adjustment ring to extend the electrode blades so that the electrode blades can enter deeper into the sample liquid.
		Add extra liquid to bring the liquid level up to the electrode tips. (Use consistent apical and basolateral volumes to make consistent comparisons.)
	The electrode tips (sensing region) may have deposits of cell culture media constituents.	The electrode tips (sensing region) require enzymatic (Tergazyme or Enzol) cleaning.
	Radio frequency is interfering with the instrument's (EVOM system's) readings.	Turn off or move any cellular phones farther away from the experimental setup.

* Defining Term:

- Drift–Readings that continuously increase or decrease significantly (either voltage or resistance) over time. Example: At 1000 Ω, the reading is increasing 100 Ω/minute. (A drift of 10 Ω/minute is acceptable.) Excessive drift may be caused

by changes in the pH or temperature, or the electrode needs cleaning. See “Cleaning/Maintaining the STX4 Electrodes” on page 9.

- Instability–At 500 Ω, the reading jumps from 450 to 550 Ω and does not settle down (an instability ±5 Ω is acceptable in the 500 Ω range). In the higher ranges, up to ±1000 Ω is acceptable at the 100K range. Electrodes showing instability may require enzymatic cleaning. See “Cleaning/Maintaining the STX4 Electrodes” on page 9.

NOTE: If you have a problem/issue that falls outside the definitions of this troubleshooting section, contact the WPI Technical Support team at (941) 371-1003 or technicalsupport@wpiinc.com.

APPENDIX A: KCL TESTING MIXTURES

To prepare solutions with different KCl concentrations, use the following table as a guide. Start by making a solution of 160 mM concentration.

Molecular weight of KCl = 74.55 g/mol

To make 100 mL of 160 mM KCl, add 1.193 gm KCl to 100 mL of distilled water.

Use	Add DI Water	Results
50 mL of 160 mM KCl	50 mL	100 mL 80 mM KCl
50 mL of 80 mM KCl	50 mL	100 mL 40 mM KCl
50 mL of 40 mM KCl	50 mL	100 mL 20 mM KCl
50 mL of 20 mM KCl	50 mL	100 mL 10 mM KCl

APPENDIX B: COMPATIBLE INSERTS AND PLATES

Corning	Millipore	Material	Membrane Diameter (mm)	Growth Surface Area (cm^2)	Membrane Pore Size (µm)
3470			6.5	0.33	0.4
3472	**PITP01250**		6.5	0.33	3.0
3413	**PCF** *Insert*		6.5	0.33	0.4
3415	**PITP01250** **PCF** *Insert*		6.5	0.33	3.0
3421			6.5	0.33	5.0
3422	**PIEP01250** **PCF** *Insert*		6.5	0.33	8.0
3495	**PIHT12R48*** **PET** *Insert*		6.5	0.33	0.4
	PIHA01250	HA *Insert*	6.5	0.33	0.45
	PICM01250	CM *Insert*	6.5	0.33	0.4
3496	**PISP12R48*** **PET** *Insert*		6.5	0.33	3.0
	PIRP12R48 **PET** *Insert*		6.5	0.33	1.0
	PIMP12R48* **PET** *Insert*		6.5	0.33	5.0
	PIEP12R48* **PET** *Insert*		6.5	0.33	8.0
	PIXP01250 **PCF** *Insert*		6.5	0.33	12
	PIHP01250				1.0
	PITT01250				3.0

* Tri-supports

Nunc	Culture Area (cm^2)	Pore Size (µm)
140620	0.47	0.4
140627	0.47	3.0
140629	0.47	8.0

ThinCert™	Membrane Material	Membrane Pore Size (µm)	Pore Density (cm^2)	Optical Membrane Properties
662640	PET	0.4	1×10^8	translucent
662641	PET	0.4	2×10^6	transparent
662610	PET	1.0	2×10^6	transparent
662630	PET	3.0	0.6×10^6	transparent
662631	PET	3.0	2×10^6	translucent
662638	PET	8.0	0.15×10^6	translucent

Millicell	Membrane Pore Size (µm)
MCHT24H48	0.4
MCRP24H48	1.0
MCSP24H48	3.0
MCMP24H48	5.0
MCEP24H49	8.0

FREQUENTLY ASKED QUESTIONS

Do the STX4 electrode blade tips tend to darken over the period of use or during storage?

Yes, it is a normal process. The **STX4** electrode tips tend to darken (cosmetic), but the electrode or the electrode blade functionally remains unaffected.

Can the STX4 electrode blade tip color be significantly different on the outer side as compared to the inner side?

Yes, it is normal (cosmetic) and can be attributed to the path of current flow between the two electrode blades. Generally, the outer side of electrode blade tips will tend to darken more since the outer electrodes are involved in applying electrical current from the EVOM (Fig. 32). The inner side of the electrode blade tips will tend to remain relatively whitish (tip color), since no electrical current passes through that side. These inner electrodes (the inner side of the electrode blade tips) are involved in the detection of change (voltage) in response to applied current. This observed difference or inconsistency of the tip color (inner side versus outer side) does not affect the actual function of the electrode blade or the electrode.

Fig. 32—The darkening of the STX4 electrode tip and a significant difference in color between inner and outer sides of the electrode blades (during storage or after a period of use) are considered normal.

NOTE: WPI performs special conditioning of all electrode blades individually (especially the tips) and verifies proper functionality of each manufactured electrode blade and **STX4** electrode.

Why am I getting dashes as reading on EVOM™ Manual, even if I have the STX4 electrode inside the sample well?

An electrode in air or partially immersed in the liquid can show dashes since it records unstable read outs. The electrode tip portion (sensing region) must stay fully immersed. You may also notice unstable read outs when the electrode tip is not fully immersed. Make sure to select apical and basolateral volumes so that the electrode tips of both electrode blades stay fully immersed.

Rotate the length adjustment ring clockwise so that the electrode blades can enter deeper into a sample and the electrode tips may be able to stay fully immersed in the liquid volumes. (See "Adjusting the Exposed Length of Electrode Blades" on page 6.)

Fig. 33—Verify the electrode sensing tips (highlighted region) on both blades stay fully immersed in a conductive liquid (cell culture media or buffer) during measurement. You need to have adequate apical and basolateral volumes to get a stable reading.

Why are the readings fluctuating while using STX4 and what should I do?

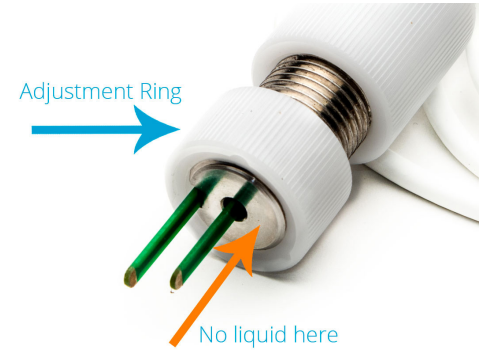

Fig. 34—Liquid on the metal surface (orange arrow) of the electrode can contribute to reading fluctuations.

Liquid in contact with the metal surface (Fig. 34, see orange arrow) can lead to reading fluctuations. Use a Kim-wipe to dry the metal surface. Avoid touching the tip. If necessary, rotate the white electrode length adjustment ring (Fig. 34, see blue arrow) so that electrode rest on top of the sample well correctly. The electrode must rest on

the white adjustment ring, not on the flat metal surface. This minimizes the chance of liquid touching or spilling onto the metal surface. If the reading still fluctuates after removing the liquid from the metal surface and after cleaning the electrode, it may be the time to replace the electrode blades with a new set of blades.

Can increasing or changing sample liquid volumes change my resistance values?

Yes. You can expect to see a change of raw resistance values. That is why you should subtract the blank values (blank *transwell* with no cells) from the sample values (*transwell* with cells). This way, you subtract the blank value with increased volume from samples with increased volume. Thus, any change of resistance contributed by increased volume is omitted. Consistently use the same volumes for all your samples in an experimental setup.

Are the electrical resistance and transepithelial electrical resistance (TEER) the same thing?

No. Multiply the measured resistance by the appropriate surface area of the membrane to calculate TEER. For example, if a 6.5 mm (24-well plate) *insert* measures 565 Ω, the TEER is:

$$565\ \Omega \times 0.33\ cm^2 = 186.45\ \Omega\text{-}cm^2$$

[For a 24-well plate (6.5 mm *insert*), area = 0.33 cm^2 and for a 12-well plate (12 mm *insert*) area = 1.13 cm^2. Please refer to the manufacturer's technical details of the specific *insert*/*transwell* (for example, Millipore, Corning, etc.) to find accurate information about the membrane area applicable to a specific *insert* part number.]

Can you suggest some experimental parameters that can be controlled to obtain more consistent TEER results?

- Temperature is known to affect TEER values. We recommend that you maintain a consistent sample temperature to obtain consistent values. We suggest taking the well plate with *inserts* containing cells out of the incubator and letting the plate stabilize at room temperature inside the laminar flow hood for 15-20 minutes before taking measurements. By this time, all the samples will be around the same room temperature.
- After adding liquids to *transwells*, wait for 15 minutes before taking measurements. The liquid levels inside and outside of the *insert* will tend to come to the same height or level and will provide better reading stability.
- We recommend using the same conductive liquid with the same ionic concentration both in the apical (top of the cell culture *insert*) and the basolateral (bottom of the *insert*) sides. For example, use the same media both inside and outside of the *insert*.
- Application of consistent volumes of the fluid (media/buffer) during all experiments reduces variability.

WARRANTY

WPI (World Precision Instruments) 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 30 days* 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.*

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