



WORLD
PRECISION
INSTRUMENTS

INSTRUCTION MANUAL

EVOM3

Epithelial Volt/Ohm Meter

Serial No. _____

www.wpiinc.com

110220

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QUICK START

Resistance

1. Power on the **EVOM3** using the power switch on the rear panel.
2. If a filename other than the default (plate1) is required:
 - Press and hold the **Store** button for 2 seconds to enter the **Store Option** menu.
 - Select **Filename** to enter a new name.
 - Press **Clear**.
 - Use the on screen keypad to enter a new name.
 - Press **Enter**.

NOTE: Auto indexing changes the name to a numeric sequence formatted with the name, a prefix and a sequential number (name_prefixN).

3. Press **Setup** to access the **Setup** menu.
 - Select Mode–Set the mode to Resistance.
 - Select Plate–Choose the plate size.
 - Display Units– Select the display units in ohms or K-ohms.
 - Resistance–Select the resistance range (auto or the desired range).
 - Calibrate–Press Calibrate to calibrate the electrode. (This uses a 10K Ω resistor to calibrate the cell ranges).
4. Press **Return** to navigate back to the main menu.
5. Insert a USB thumb drive into the USB port on the side of the **EVOM3** and begin measuring by pressing the foot switch or touching the **Store** button on the screen to store and advance to the next well. Tap the **Next Well** area on the right side of the screen to advance in increments of one well without taking a measurement. Repeat the sampling process.
6. Once the last well has been recorded, the file storing selection notification appears. Press **Store**. A system message will report if the USB thumb drive is usable, or it will display an error. If the USB thumb drive is not plugged in or it is not recognized, a warning message will appear. Press **Cancel** and try another drive. The **Store** function cannot be used on an unrecognized drive. **EVOM3** should respond with "File saved" within 15 seconds. If "Saving file..." is still seen after 20 seconds, press the **Return** key and use another USB drive. Once a file has been saved, open the file on a computer to verify the contents.

NOTE: The file saving routine can take up to 15 seconds to complete. See the USB cautions on page 5.

7. Remove the USB thumb drive and transfer the data file to your computer as a CSV file (Excel).

Voltage

1. Power on the **EVOM3** using the power switch on the rear panel.
2. If a filename other than the default (plate1) is required:
 - Press and hold the **Store** button for 2 seconds to enter the **Store Option** menu.

- Select **Filename** to enter a new name.
- Press **Clear**.
- Use the on screen keypad to enter a new name.
- Press **Enter**.

NOTE: Auto indexing changes the name to a numeric sequence formatted with the name, a prefix and a sequential number (name_prefixN).

3. Press **Setup** to access the **Setup** menu.
 - Select Mode–Set the mode to PD.
 - Select Plate–Choose the plate size.
 - Display Units– Select the display units in volts or millivolts. (The normal mode is millivolts.)
 - Calibrate (zero) the electrodes in saline. Press the **Probe Null** button. Push the **Null** button. Wait for the second message stating “Solution value 0.0” before returning. This action removes any voltage offsets on the electrodes.
 - Press **Return** to navigate back to the main menu.
4. Insert a USB thumb drive into the USB port on the side of the **EVOM3** and begin measuring by pressing the foot switch or touching the **Store** button on the screen. Tap the **Next Well** area on the right side of the screen to advance to the next well. Repeat the sampling process.
5. Once the last well has been recorded, the file storing selection notification appears. Press **Store**. A system message will report if the USB thumb drive is usable, or it will display an error. If the USB thumb drive is not plugged in or it is not recognized, a warning message will appear. Press **Cancel** and try another drive. The **Store** function cannot be used on an unrecognized drive. **EVOM3** should respond with “File saved” within 15 seconds. If “Saving file...” is still seen after 20 seconds, press the **Return** key and use another USB drive. Once a file has been saved, open the file on a computer to verify the contents.

NOTE: The file saving routine can take up to 15 seconds to complete. See the USB cautions on page 5.
6. Remove the USB thumb drive to transfer the data file to your computer as a CSV file (Excel).

TEER Calculations

To compute TEER, multiply the measured resistance by the surface area's listed below. For example, a 12 mm insert measures 565 Ω, the TEER is $565 \Omega * 1.13$ or 638 Ω.

- 6 well plate (24 mm inserts) 4.52 cm²
- 12 well plate (12 mm inserts) 1.13 cm²
- 24 well plate (6.5 mm inserts) 0.3316 cm²
- 96 well plate (4.3 mm inserts) 0.145 cm²

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 or data. 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—EVOM3

INTRODUCTION

EVOM3 is the WPI's newest instrument to measure Trans Epithelial Electrical Resistance (TEER) measurement. The **EVOM3** has been re-engineered to facilitate ease of accurate, repeatable measurements and transfer of data. You no longer need to log data by hand. The resistance or voltage information is written to a USB drive in a format (CSV) that can be opened by most spreadsheet programs on either PC or Mac. The processor controlled instrument is more stable and accurate. Included is a footswitch for quick hands free recording to the USB drive. The touch screen interface is easy to use and setup for setting control wells in resistance or voltage modes and measurements. The auto ranging resistance feature allows for fast resistance measurements, and an overrange display feature eliminates false readings. The **EVOM3** now has adjustable current levels in three fixed ranges with two lower ranges for sensitive membranes and high resistance ranges up to 100K Ω .

The **STX2-PLUS** electrode was redesigned for easier insertion into and 24-well plates and to be location re-placeable in the insert for repeatable and consistent measurements. The new shielded electrodes are now designed to minimize electrical interference and to be more easily maintained.

Confluence of a cellular monolayer is determined by an increase or a plateau in tissue resistance detected using the unique electronic circuit of the **EVOM3** and the new

STX2-PLUS electrode. The **EVOM3** qualitatively measures cell monolayer health and quantitatively measures cell confluence. The **EVOM3** produces a low AC current that avoids electrode metal deposits and adverse effects on tissues which can otherwise be caused by higher DC currents. The **EVOM3** uses low current and voltages and is designed for non-destructive testing for epithelial monolayer confluence in cell cultures. In addition, resistance readings are unaffected by membrane capacitance or membrane voltage. The accuracy and repeatability of the **EVOM3-STX2-PLUS** system makes this instrument ideal for permeability, PD and other detailed membrane studies.

EVOM3 Features

- Low noise design offers greater resolution and accuracy
- Automatic 20X sample averaging improves accuracy and stability
- Adjustable fixed measurement currents (2, 4 or 10 μA)
- Resistance auto ranging from 1 Ω to 100,000 Ω or with three fixed current ranges
- Reliable low current, low voltage design prevents metal ion transport
- Fast resistance stabilization on low levels under 200 Ω with resolution to 0.1 Ω
- Ergonomic tilt stand for low glare operation
- Graphical display of popular plates (6, 12, 24, 96) for trend analysis
- The display shows the most recent set of parameters
- Automatic plate indexing operation with or without control well subtraction for resistance and potential difference (PD) measurements
- Continuous data logging via USB (PC, Mac, Linux)
- Saves date stamped data to a spreadsheet readable file on a USB drive
- Upgradable firmware

EVOM3 Benefits



Eliminates errors and reduces experimental processing time



Auto data logging eliminates the need to track data by hand



The small footprint allows more bench space



Easy calibration and verification



Footswitch for hands-free recording



Prevent data loss with auto save and data recovery when battery is low



TEER is easily computed by applying a unit area formula to the resistance

STX2-PLUS Features

- **STX2-PLUS** new electrode designed for 12 and 24-well plates.
- Weighted self-standing electrode for hands free stable measurements
- Shielded cable to minimize electrical and cell phone interference

STX2-PLUS Benefits

- Keyed electrode base for repeatable placement gives more consistent results eliminating the need for multiple readings.
- Easy to maintain

Notes and Warnings

Choice of current/range: The **EVOM3** has three ranges of current from 2 μA to 10 μA and each current range has its own resistive upper limit up to 100K Ω :

- 10K Ω range = 10 μA
- 50K Ω range = 4 μA
- 100K Ω range = 2 μA

The auto range will adjust the current as needed from 2 μA to 10 μA depending on the resistance.

General rules

- The higher the current the greater the stability of the measurement. The fixed ranges may be more consistent with a fixed current for some cell lines.
- If a cell line is not expected to read above 10K Ω , then choose that fixed range.

Use of the charger: The **EVOM3**'s Li-ion battery will last 8 hours on its own. The charging time is 5.5 hours with the unit powered off. The **EVOM3** charger should not be plugged in all the time. The charger may be used to power the **EVOM3** for normal and long-term use.

NOTE: In the higher resistance ranges (50K -100K) there may be a slight instability added from the noise of the power supply, in that case unplug it during those measurements.

USB Drives: WPI has supplied a tested USB thumb drive. Most drives of 32 Gb (USB2.0) or smaller sizes have been tested.



CAUTION: Not all USB drives work on the **EVOM3**.

To test a USB drive, see "Sample Resistance File (*.CSV)" on page 16. This test saves a file to the drive and reads it back. A system message will report if the USB thumb drive is usable, or it will display an error. If the USB thumb drive is not plugged in or it is not recognized, a warning message will appear. Press **Cancel** and try again. The **Store** function cannot be used on an unrecognized drive. **EVOM3** should respond with "File saved" within 15 seconds. If "Saving file..." is still seen after 20 seconds, press the **Return** key and use another USB thumb drive. Once a file has been saved, open the file on a computer to verify the contents.

Tips and Advice

Out of Range Error

If you see ---- (5 dashes) on the main resistance display, then the resistance reading is out of range. Check the ohms range setting to see if it can be increased to display a higher resistance or set it to **Auto**. If the display still shows ----, then go to the **Blank Handling** screen and check the settings and the reading. A saved reading will show a space (" ") in the grid if the display shows out of range. Do not forget to reset the blank to zero or go back to a control well resistance when you are finished.

NOTE: The 100K Range can read 5% over to 105K Ω .

Zeroing the blank handling value

1. Select **Setup**.
2. Then, press **Short Electrodes**.
3. Press the **Blank Handling** button.
4. Set the reading to zero.
5. Return to the **Setup** page and open the electrodes to remove the short.

Zeroing the probe null offset

1. Select **Setup**.
2. Then, press **Short Electrodes**.
3. Press the **Probe Null** button.
4. Set the reading to zero.
5. Return to the **Setup** page and open the electrodes to remove the short.

Resistance levels that are unexpectedly lower or higher can be attributed to a number of things besides an incompletely grown membrane. Any of these items can change the resistance reading:

NOTE: Many of these conditions are eliminated with the **STX2-PLUS** electrodes.

- Electrode resistance (Keep the electrodes clean and chlorided.)
- Fluid levels (Try to maintain the same volume for every insert. A variation of 50 μL can have a large effect in a small insert.)
- Changing CO_2 levels and temperature drift can account for large changes in the resistance reading. The use of a warming plate can reduce this effect.
- We recommend that both fluid levels are at the same height so that you do not have any pressure differentials and the apical well is filled first so that you do not dislodge the membrane from the filter by hydrostatic pressures. If the membrane detaches itself, then it was not adhering to the inserts filter. A small gap from dislodged tissue at the edge of the membrane can open a clear path for the **EVOM3** to read a lower resistance. Instead of the current going through the membrane, the electricity is going around the membrane. Electricity will take the path of least resistance and the **EVOM3** will measure that as a lower resistance.

NOTE: Cleaning the electrode often corrects most of these issues. See "Maintenance" on page 26.

In the **STX2-PLUS** manual is a KCl molarity test. This test will demonstrate electrode functionality.

Charging the EVOM3

The **EVOM3** battery may also be charged with a Mini-B USB cable P/N 803026 (not supplied).

Parts List

After unpacking, verify that there is no visible damage to the sensor. Verify that all items are included:

- (1) **EVOM3** Epithelial Volt Ohm Meter
- (1) **STX2-Plus** Electrode set
- (1) **300749** USB drive 32 GB (For storage and this contains a Python 3.8 program for continuous digital monitoring of a target insert. See page 36.)
- (1) **503535** USB cable
- (1) **99673** 1000Ω Test Resistor
- (1) **803025** A/C power cord and charger
- (1) **13142** Foot switch

Instruction Manuals for the **EVOM3** and **STX2-Plus** electrode can be downloaded at <https://www.wpiinc.com/manuals>.



Fig. 2—The EVOM3 includes these parts.

NOTE: An **99672** EVOM2 to EVOM3 Electrode Adapter is sold separately. The STX2, STX3 and all STX100s require the use of this adaptor with the **EVOM3**. See "Accessories" on page 28.

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 47 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 (4") of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 47 of this manual.

INSTRUMENT DESCRIPTION

EVOM3 Meter



Fig. 3—USB port, Electrode connection



Fig. 4—Foot switch, Future expansion, reset, power connection, power switch

STX2-PLUS Electrodes

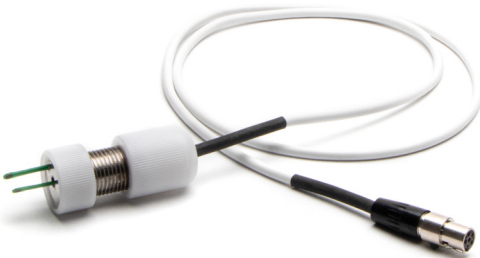


Fig. 5—STX2-PLUS electrode

When using the electrode with a 24-well plate, WPI recommends the following fluid levels:

- Apical well – 250 μ l
- Basal well – 750-1000 μ l (when using a Corning 347x plate).

Place the electrodes into the well so the outer tip passes between the outer edge of the insert and the inner well of the insert. Release the electrode so that it stands free, then after a few seconds record the reading. Slide the cable on the table toward the plate and electrode to prevent tipping.

Theory of Operation

The **EVOM3** system uses a four electrode sensor, two for current sourcing and two for voltage measurement.

The **EVOM3** passes a known constant current through the membrane on two electrodes and measures the voltage needed to pass that current on the other two electrodes and computes resistance using Ohms law. The polarity of the current changes from positive current to negative current 12.5 times per second. This polarity change avoids leaving a charge on the membrane and negates any voltage offsets due to the membrane potential or from the electrodes. The amount of current and voltage is low (2, 4 or 10 μ A) to minimize any tissue stimulation and to prevent the migration of metal ions.

Main Display

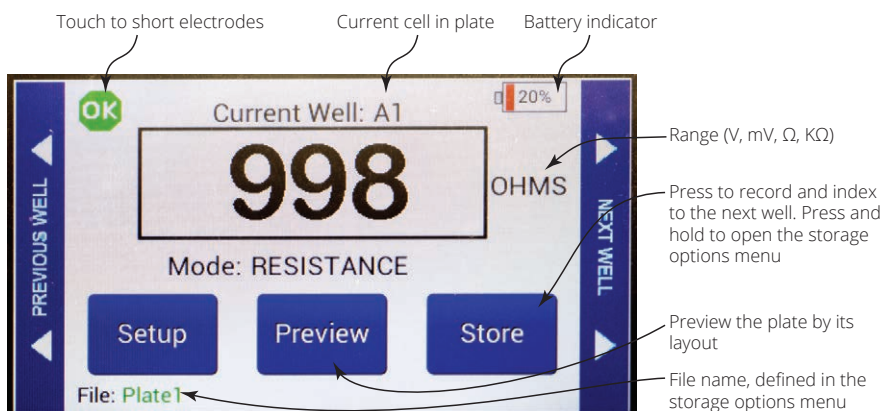


Fig. 6—The main EVOM3 display.

A negative resistance number will appear in red text. This can be caused by a faulty electrode in media, (see "Troubleshooting" on page 29) or a blank setting that is over the measurement (see "Blank Handling Page" on page 13).

Setup Page

Press *Setup* on the main screen to open the *Setup* page.

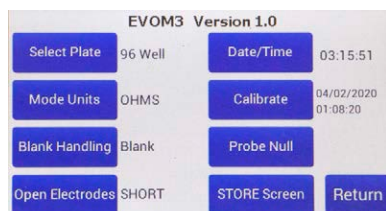


Fig. 7—Setup page

Select Plate – Select the number of wells and the sampling pattern.

Mode Units – Choose the Units for sampling. (Options include Millivolts, Volts, Ohms and K Ohms.)

Blank Handling – Lets you subtract the control blank from the current resistance measurement.

Short Electrodes – Shorts V_1 - V_2 and is used to nullify the galvanic charge in the electrodes when the electrodes are placed in a saline solution for 4–8 hours to remove the charge. See also "Probe Null" on page 14.

Date/Time – Select the Date and Time formats for your data files.

Calibrate – Calibrate the electrode. (This uses a 10K Ω 0.1% resistor.) See "Instrument Diagnostics and Calibration" on page 21.

Probe Null – Neutralize the offset charge on V_1 - V_2 .

Store Screen– Store data files, configure how the files are named and if they are automatically numbered.

Plate Selection Page and Plate Preview

1. Press *Select Plate* on the *Setup* page to access the *Plate Selection* screen.

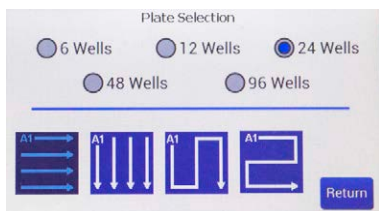


Fig. 8—Choose the number of wells and the sampling pattern.

2. Select the radio button for the number of wells in your plate.

3. Select the sampling pattern from the options in the bottom half of the window.

- Press **Return** to save the setup. When you press **Preview** from the main screen you will see the plate preview files like the samples below.

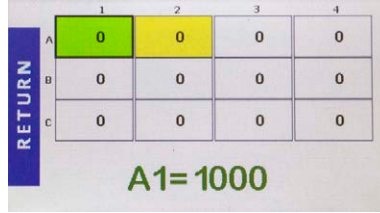
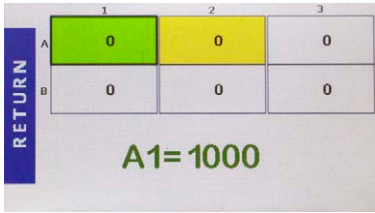


Fig. 9—(Left) 6-well plate preview

Fig. 10—(Right) 12-well plate preview

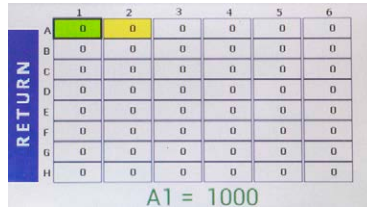
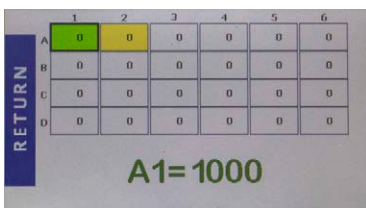


Fig. 11—(Left) 24-well plate preview

Fig. 12—(Right) 48-well plate preview

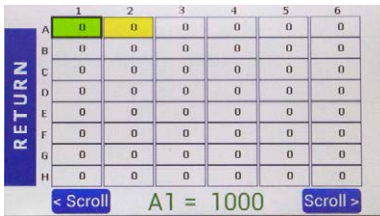


Fig. 13—96-well plate preview. (Press scroll to see the additional wells.)

NOTE: An out of resistance range will display ---- (5 dashes) on the screen. Adjust the range to correct the error. A saved well under preview will show a space (" ") if the display was recorded out of range. The 100K range can read 5% over the 105K Ω.

Mode Units

- Press **Mode Units** on the **Setup** page to access the **Select Volts or Ohms** screen.

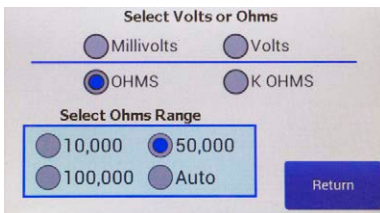


Fig. 14—Choose the unit of measurement and (if you are using Ohms) the range.

- Choose your units. Options include Millivolts, Volts, Ohms and K Ohms. If you choose ohms, then you must also select an Ohms Range.

Fixed Current	
10 K	10 μ A
50 K	4 μ A
100 K	2 μ A

Variable Current	
Auto	2 μ A to 10 μ A

- Press *Return*.

Blank Handling Page

- Press *Blank Handling* on the *Setup* page to subtract the control well blank from the current resistance measurement.

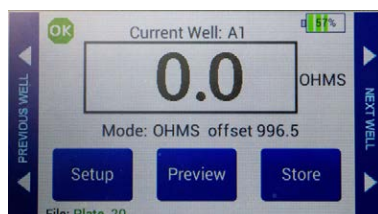
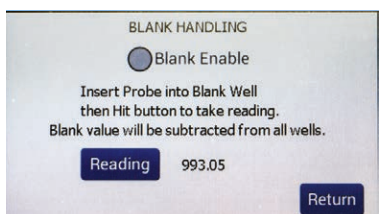


Fig. 15—(Left) Blank Handling window.

Fig. 16—(Right) The main screen shows the offset underneath the reading.

- Insert the probe into an empty well filled with media. Press *Reading* to make a measurement.
- Select the *Blank Enable* radio button to enable the offset.
- Press *Return*. The resistance offset shows under the reading on the main screen. If the blank (offset) is greater than the resistance reading, the reading will display negative numbers in red text. The saved data file reflects the subtraction.

NOTE: This includes fluid and electrode resistances.

Setting the Date and Time

- Press *Time/Date* on the *Setup* page to set the date and time format.

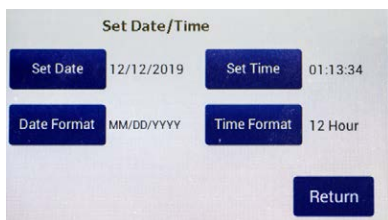


Fig. 17—The Set Date/Time screen lets you set the date and time and choose the display format.

- Press *Set Date*. Set the appropriate date and press *Return*.

3. Press *Date Format*. Choose your preferred format and press *Return*.

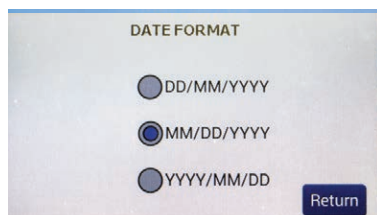


Fig. 18—(Left) Set the date and press *Return*

Fig. 19—(Right) Choose the date format and press *Return*

4. Press *Set Time*. Set the appropriate time and press *Return*.
5. Press *Time Format*. Choose 12 hour or 24 hour and press *Return*.

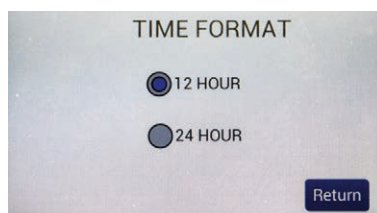


Fig. 20—(Left) Set the time and press *Return*

Fig. 21—(Right) Choose the time format and press *Return*

Probe Null

1. Press *Probe Null* on the *Setup* page to access the *NULL Probe Process* screen.

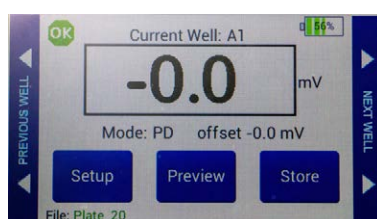
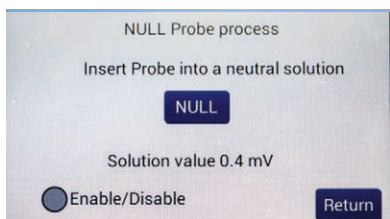


Fig. 22—(Left) Null probe process.

Fig. 23—(Right) The potential difference offset shows on the main screen under the reading.

2. Place the electrodes in a saline media.
3. Push the *NULL* button. Wait for the second message stating "Solution value 0.0" before returning. This action removes any voltage offsets on the electrodes and neutralize the offset charge on V_1 - V_2 .
4. Select the *Enable/Disable* radio button to use the offset.
5. Press *Return*. The potential difference offset shows on the main screen under the reading. An electrode check should show 0.0 mV. The saved data file reflects the offset.

Storing Data

1. Press and hold the **Store** button on the main screen for 2 seconds or press *Store Screen* on the *Setting* screen to configure your stored data files.



Fig. 24—Use the Store Options screen to save a data file.

2. Press *New Plate* to clear any recorded data on the preview screen and from memory.



CAUTION: This will clear any readings saved in the preview grid.

3. Press *File Name* to enter a unique name for your data file. In the entry page, select *Clear*, then type the file name, and press *Enter* when you finish. If you choose to use your file name, auto-indexing must be disabled.



Fig. 25—Press *Clear*. Type the desire name. Press *Enter*.

4. If you prefer to use the *Auto Index* function, press *Store Prefix* to enter a unique file name that will be appended with sequential numbers each time your file is saved. Select the *Auto Index* radio button to enable the *Store Prefix* and *Auto Index* buttons.
5. Press *Auto Index* if you want the **EVOM3** to automatically number the new data files sequentially. Auto indexing changes the name to a numeric sequence formatted with a prefix and a sequential number (prefixN).
6. Press **Store Now** to save the current data set. There is a time out if the USB write fails. If this error occurs, the “Saving file...” notice will not change to “File saved.” A file is typically saved in less than 20 seconds. If your file does not save, press *Return* and try using a different USB drive.
7. Press *Return*.

Viewing Data in Microsoft Excel or Other Spreadsheet Program

1. Remove the USB thumb drive from the **EVOM3** and plug it into a free USB slot in your computer.
2. Open the drive and look for files named *PlateX.CSV*.
3. If your computer has Microsoft® Excel or another spreadsheet program the file will open by clicking on the file. The well data is loaded into spreadsheet cells.
4. To transfer the data file to your computer, select the plate files and copy it to a folder on your computer.

Sample resistance file shows:

- Date, time, plate size
- **EVOM3** firmware revision
- Measurement mode with a blank or a voltage offset
- Units ohms or kilo ohms
- Recording direction, 1-4 (See "Plate Selection Page and Plate Preview" on page 11.)
- Resistance scale (10K, 50K, 100K), and the last used scale is shown
- Well data in the plate grid with the blank offset applied.

Sample Resistance File (*.CSV)

Date 03/12/2020 13:11:56 Well Plate 24

EVOM3 Version 1.0

MODE OHMS offset 999.8

Units: OHMS

Direction 1

Resistance Scale 50k Ohms

0	1	2	3	4	5	6
A	19015	19015	19015	19015	19015	19015
B	19016	19016	19016	19016	19016	19016
C	19016	19016	19016	19016	19016	19014
D	19014	19014	19014	19014	19014	19017

Sample Voltage File (*.csv)

Date 03/12/2020 13:12:48 Well Plate 24

EVOM3 Version 1.0

MODE PD offset -0.0 mV

Units: mV

Direction 1

Resistance Scale 50k Ohms

0	1	2	3	4	5	6
A	0.0	0.0	0.0	0.0	0.0	0.0
B	0.0	0.0	0.0	0.0	0.0	0.0
C	0.0	0.0	0.0	0.0	0.0	0.0
D	0.0	0.0	0.0	0.0	0.0	-0.0

USB Testing

From the *Store Options* page you can test a USB thumb drive to verify that it can be used. This drive must be formatted as FAT32.

1. Insert the drive to see if it is recognized by the **EVOM3**.
2. From the main page, press the *Settings* button. Press the *Store* button. Then, press the *USB Test* button.

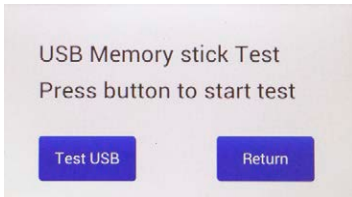


Fig. 26—Press the *Test USB* button to run the test.

3. Press the *Test USB* button to run the test or *Return* to abort the test. The USB drive will be written to with 100 lines of data to a file named USBTEST.txt and those lines will be read back. If the drive is usable a message indicates that the USB thumb drive can be used, otherwise an error is displayed.

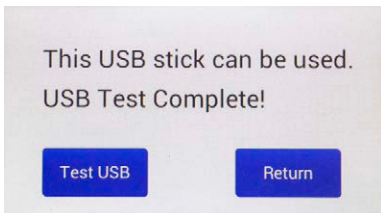


Fig. 27—(Left) The USB thumb drive passed the test and is suitable for use with the EVOM3.

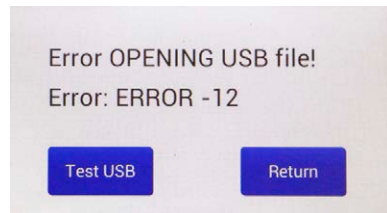


Fig. 28—(Right) The USB thumb drive is not suitable for use with the EVOM3.

4. Insert another USB thumb drive and press the *Test USB* button to test another drive or press *Return* to go back to the *Store Options* page.

SYSTEM MESSAGES

Here are some warning messages you may see.

Last Cell Warning

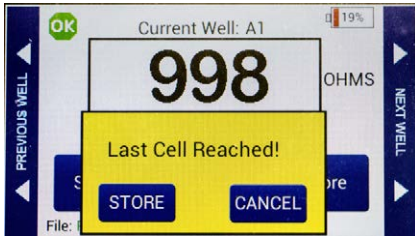


Fig. 29—After the last cell measurement is taken, this warning appears.

This is the indication that the last well in the plate has been reached and the file needs to be stored.

- Press **Store** to save using the current file name or the prefix# file name.
- Or, press **Cancel** to change the file name. Go to the **Store Options** page and change the name. Then, click **Save Now**.

Power Level Messages

When the battery level falls below 5%, a warning message appears.

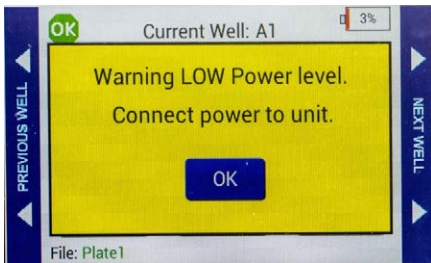


Fig. 30—The battery level is below 3%.

When the battery power level falls to 1%, a warning message appears. Save your data file. The data is saved to a backup file named PWRFAILDATA.TXT.

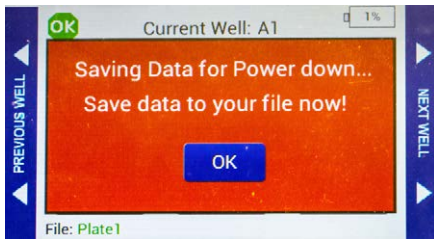


Fig. 31—When the battery level falls to 1%, you are asked to save your data file.

When the battery level falls to 0%, the system begins a 60 second countdown before powering off.

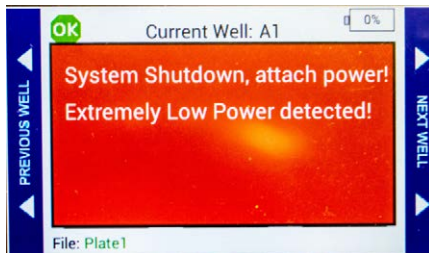


Fig. 32—When the battery power falls to 0%, a warning displays briefly.



Fig. 33—The 60 second countdown was initiated when the battery power fell to 0%.

Five seconds before shutdown, a final warning appears.

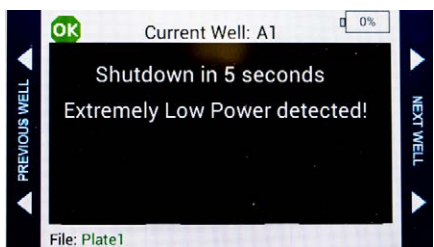


Fig. 34—(Left) When the countdown reaches 5 seconds, the final warning appears.

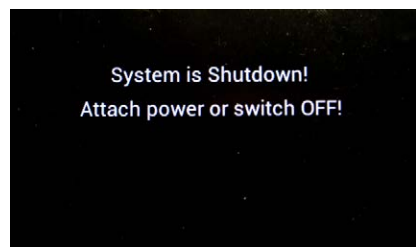


Fig. 35—(Right) Immediately before the system powers off, the shutdown message displays.

Reloading a Backup File after Power Failure

1. When power is restored a green message appears. Press **OK**.

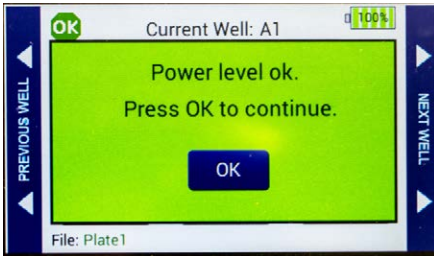


Fig. 36—(Left) Power level is restored when the unit is plugged into a power source.

2. Cycle power on the **EVOM3** by turning it off and on again. This will automatically load the backup file which was saved when the power failed. The backup file is deleted from the USB drive when you press **OK**. Then, press **Preview** to check the data. Press **Save** to store the data.

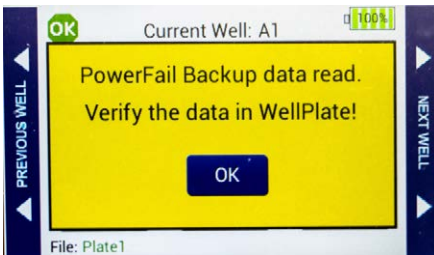


Fig. 37—The backup file is restored on power up.

OPERATIONS


IMPORTANT! Before you start making measurements, WPI recommends that you:

- Charge the **EVOM3** battery or run under mains power.
- Clean and chloride the electrodes. See "Cleaning/Maintaining the STX2-PLUS Electrodes" on page 26.
- If it is new, test the USB thumb drive. See "Sample Resistance File (*.CSV)" on page 16.
- Validate the **EVOM3** using 1000 Ω test resistor or calibrate the **EVOM3**.


Instrument Diagnostics and Calibration

WPI recommends that **EVOM3** be put through the diagnostics described below before using it for the first time and then periodically thereafter. If there is a concern that the meter or the electrode is not functioning properly, the following protocols may be used to confirm the **EVOM3** operating status.

Testing the EVOM3 Meter (Resistance) and Calibration

1. Insert the **99673** test resistor into the electrode port on the meter's right side.
2. On the main display, select the *Setup* page. In the *Mode units* area, select *Ohms* and 10,000. Press *Return*.
3. Verify that the blank handling is disabled (Setup>Blank Handling). The screen should display 1000 $\Omega \pm 1 \Omega$. If it does not, select *Setup*, push the *Calibration* button and wait for the process to complete. (This process takes 30 seconds to complete.) When the date and time are displayed, the calibration process is complete.
4. Press *Return* to access the main page. The display should show 1000 $\Omega \pm 1 \Omega$.
5. Press the *OK* in the upper left of the octagonal icon on the main display page to short the electrodes. Verify that the **EVOM3** reads 0 $\pm 1 \Omega$.
6. To return to normal **EVOM3** operation, unplug the test resistor and press the  button (upper left).

Testing the EVOM3 Meter (Voltage)

1. On the main display, select the *Setup* page. Press the *Short Electrodes* button.
2. In the *Mode units* area, select *Millivolts*. Press *Return*.
3. Press the *Probe Null* button and then *Disable*. Press *Return* twice. The main display should show 0.0 mV. If it does not, verify that the probe null is not active and that it has a 0.0 V offset, ± 0.1 mV.
4. Press the  button (upper left) to remove the electrode short.

Test Resistor "Calibration Kit"

The **99673** test resistor is supplied to verify the calibration.

Making Resistance Measurements

1. Power on the **EVOM3** using the power switch on the rear panel.
2. If a filename other than the default (plate1) is required, choose whether you will use a prefix and auto-indexing or a unique file name:
 - Press and hold the **Store** button on the main screen for 2 seconds or press **Store Screen** on the **Setting** screen to configure your stored data files.



Fig. 38—Store options screen.

- To use a prefix and auto-indexing, activate the **Auto Index** radio button. Then, press **Store Prefix** to enter a file name. Or, to use a unique file name, deactivate the **Auto Index** radio button. Then, select **File Name** to enter a new name.



Fig. 39—Keyboard to enter a unique file name.

- Press **Clear**.
- Use the on screen keypad to enter a new name.
- Press **Enter**.

NOTE: Auto indexing changes the name to a numeric sequence formatted with the name, a prefix and a sequential number (name_prefixN).

3. Press **Setup** to access the **Setup** menu.



Fig. 40—Setup page

- **Select Plate**—Choose the plate size.
- **Select Mode Units** – Choose OHMS or KOHMS for making resistance measurements. Select the resistance range (auto or 10,000 ohms).
- **Calibrate**—Press Calibrate to calibrate the electrode. (This uses a 10 K Ω 0.1% resistor). See "Instrument Diagnostics and Calibration" on page 21.
- **Blank Handling** – To subtract a blank well (including the fluid and electrode resistances), place the electrode in a blank well and press *Reading*. Press the *Blank Enable* radio button to subtract the blank value.

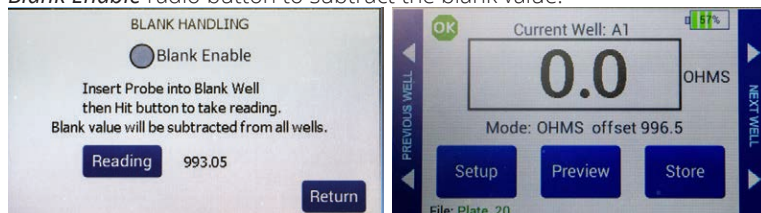


Fig. 41—(Left) Blank Handling window.

Fig. 42—(Right) The main screen shows the offset underneath the reading.

- Press *Return* to navigate back to the main menu.
4. Insert a USB drive into the USB port on the side of the **EVOM3** and begin measuring by pressing the foot switch or touching the *Store* button on the screen. Tap the *Next Well* area on the right side of the screen to advance to the next well. Repeat the sampling process.
 5. Once the last well has been recorded, the file storing selection notification appears.



Fig. 43—Warning that the last cell measurement was taken.

6. Press *Store*. A system message will report if the USB thumb drive is usable, or it will display an error. If the USB thumb drive is not plugged in or it is not recognized, a warning message will appear. Press *Cancel* and try another drive. The *Store* function cannot be used on an unrecognized drive. **EVOM3** should respond with "File saved" within 15 seconds. If "Saving file..." is still seen after 20 seconds, press the *Return* key and use another USB drive. Once a file has been saved, open the file on a computer to verify the contents.

NOTE: The file saving routine can take up to 15 seconds to complete. See the USB cautions on page 5.

7. Remove the USB thumb drive to transfer the data file to your computer as a CSV file (Excel).

Making Voltage Measurements

1. Power on the **EVOM3** using the power switch on the rear panel.
2. If a filename other than the default (plate1) is required:
 - Press and hold the *Store* button on the main screen for 2 seconds or press *Store Screen* on the *Setting* screen to configure your stored data files.



Fig. 44—Store options screen.

- To use a prefix and auto-indexing, activate the *Auto Index* radio button. Then, press *Store Prefix* to enter a file name. Or, to use a unique file name, deactivate the *Auto Index* radio button. Then, select *File Name* to enter a new name.



Fig. 45—Keyboard to enter a unique file name.

- Press *Clear*.
- Use the on screen keypad to enter a new name.
- Press *Enter*.

NOTE: Auto indexing changes the name to a numeric sequence formatted with the name, a prefix and a sequential number (name_prefixN).

3. Press *Setup* to access the *Setup* menu.



Fig. 46—Setup page

- **Select Plate**—Choose the plate size.
- **Select Mode Units** – Choose volts or millivolts (the normal mode is millivolts).

- **Electrode Equilibration** – Zero the electrodes' galvanic charge in saline.
- Press the **Probe Null** button. Push the **NULL** button. Wait for the second message stating "Solution value 0.0" before returning. This action removes any voltage offsets on the electrodes. Then activate the **Enable/Disable** radio button to offset the electrode potential.

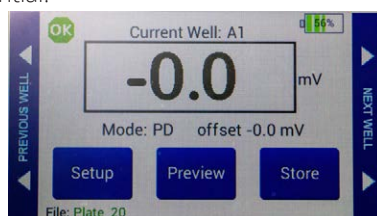
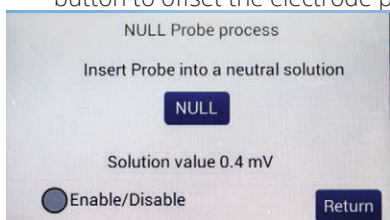


Fig. 47—(Left) Null probe process.

Fig. 48—(Right) The potential difference offset shows on the main screen under the reading.

- Press **Return** to navigate back to the main menu.
4. Insert a USB drive into the USB port on the side of the **EVOM3** and begin measuring by pressing the foot switch or touching the **Store** button on the screen. Tap the **Next Well** area on the right side of the screen to advance to the next well. Repeat the sampling process.
 5. Once the last well has been recorded, the file storing selection notification appears.



Fig. 49—Warning that the last cell measurement was taken.

6. Press **Store**. A system message will report if the USB thumb drive is usable, or it will display an error. If the USB thumb drive is not plugged in or it is not recognized, a warning message will appear. Press **Cancel** and try another drive. The **Store** function cannot be used on an unrecognized drive. **EVOM3** should respond with "File saved" within 15 seconds. If "Saving file..." is still seen after 20 seconds, press the **Return** key and use another USB drive. Once a file has been saved, open the file on a computer to verify the contents.

NOTE: The file saving routine can take up to 15 seconds to complete. See the USB cautions on page 5.

7. Remove the USB thumb drive to transfer the data file to your computer as a CSV file (Excel).

MAINTENANCE

Electrodes must be properly cleaned, sterilized and stored.



CAUTION: Do not flame electrodes. Doing so will cause them to melt.

Cleaning/Maintaining the STX2-PLUS Electrodes

IMPORTANT! With the use of DMEM, the electrode surface will become coated with protein or other foreign materials. This build-up, or contamination, will degrade the performance of the system. After every use, rinse the STX electrodes with distilled water and store them dry. Periodically clean your STX electrodes with Tergazyme, Enzol or Endozyme proteolytic detergents. (These detergents are manufactured by Alconox, Johnson & Johnson, Ruhof.)

1. Rinse with the electrodes with distilled water and dry them.
2. Make a 1% solution of Tergazyme according to the manufacturer's instructions.
3. Suspend the tips of the electrodes in the Tergazyme solution, with the exposed electrode surfaces fully immersed. During soaking, you can brush the surfaces of the electrodes with a soft brush (like a toothbrush), if desired. The soaking time varies according to your maintenance schedule and the frequency of your cleaning.
 - Soak the electrodes overnight when the electrodes have not been on a routine maintenance cleaning schedule.
 - Soak 60 minutes if you are on a weekly cleaning schedule.
 - Soak 10-30 minutes if you clean your electrodes daily.
4. Rinse the electrodes well with distilled or de-ionized water. Allow them to air dry and store the electrodes dry away from exposure to sunlight.

Disinfecting the STX2-PLUS Electrodes

The **STX2-PLUS** electrodes are resistant to most methods of low temperature chemical disinfection. A solution of 5% sodium hypochlorite (undiluted household bleach) is a good choice (WPI# tho-phthaladehyde (Cidex OPA or Rapicide OPA), 30/70 ethanol or 30/70 isopropyl alcohol are also acceptable.

The **STX2-PLUS** electrodes require a 10-15 minute soak in a solution of 5% sodium hypochlorite (undiluted household bleach) to revitalize the electrodes.




CAUTION: NEVER leave the electrode in alcohol for more than 30 minutes at a time. Continuously soaking the electrode in alcohol will weaken the protective coating on the electrode and shorten its life. A damaged electrode showing peeling of the transparent coating will expose tissues to copper. An electrode showing signs of peeling must be replaced.

 **CAUTION:** Ammonia is NOT recommended, because silver chloride dissolves in ammonia.


Sterilizing the STX2-PLUS Electrode


The **STX2-PLUS** electrodes are non-sterile as supplied. Acceptable low temperature sterilization methods for the electrodes include gamma irradiation and ethylene oxide gas (ETO).

 **CAUTION:** Do NOT autoclave the electrode.

 **CAUTION:** Do NOT use ionized hydrogen peroxide (H₂O₂).

 **CAUTION:** Do NOT expose the electrodes to ultra-violet light, because UV light decomposes silver/silver chloride electrodes.

 **CAUTION:** Do not sand or abrade the electrode surfaces. A damaged electrode will expose tissues to copper. An electrode showing deep scratches on the Ag-AgCl surfaces must be replaced.


 **CAUTION:** Do not immerse the electrode head.

Rechloriding the Electrodes

The **STX2-Plus** electrode must be dipped into chlorine bleach (5% sodium hypochlorite - WPI# **TEER-CL**) on occasion to maintain the amount of Ag to AgCl balance. Otherwise, the electrode will become unstable. A 10–15 minute immersion of the tips is recommended before each daily use. (These treatments may be reduced to once per week as experience guides). DMEM deposits cause resistance measurements to become unstable and the coating appears as a dark crust on the electrode surfaces. See "Appendix A: KCL Testing Mixtures" on page 35. A properly chlorided silver surface will appear to be a dark silvery grey coloration.

<https://www.wpiinc.com/wp-34521-endozime-enzymatic-detergent-169-oz>

<https://www.wpiinc.com/7363-4-enzol-enzymatic-detergent-1-gallon>

 **CAUTION:** Too much chloriding can also lead to instabilities. A short 5 minute soak in ammonia water (3%) will convert the AgCl back to Ag. After a DI water rinse, repeat the chloriding. Follow this with an electrode test.

Storing the Electrodes

After a daily use, wash and store the electrodes dry.

Testing the STX2-PLUS Electrodes

If another electrode is being used, refer to that electrode's manual.

To make sure the electrode is operating properly, a resistance test in KCl is used. This test demonstrates electrode functionality.

40 mM KCl	80 mM KCl	160 mM KCl
38-66 Ω	27-44 Ω	12-20 Ω

At the lower molarities (10–20 mM), a DMEM coated electrode will begin to show instabilities at the higher concentrations. If the resistance readings in the KCl mixtures are over 10% out of range, then the electrode may need cleaning. See "Cleaning/ Maintaining the STX2-PLUS Electrodes" on page 26. An Excel scatter plot of the molarity vs. resistance (as a power) should show an R^2 value $> 0.99x$.

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) should show an R^2 value $> 0.99x$. 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 mixtures are out of range, then the electrode may need cleaning. See "Cleaning/ Maintaining the STX2-PLUS Electrodes" on page 26. 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 for longevity.**

ACCESSORIES

WPI Part # Description

EVOM3 ACCESSORIES & REPLACEMENT PARTS

99672	EVOM3 legacy electrode Adaptor to use EVOM3 with EVOM2 electrode
99673	EVOM3 Calibration Kit 1000 Ω 0.1% Test Resistor
99675	EVOM2 Electrode adaptor to use EVOM2 with EVOM3 electrodes
99916	EVOM3 EndOhm cable
803026	EVOM3 upgrade cable USB Mini-B
300749	USB drive 32GB, programmed
503535	USB2 cable
803025	EVOM3 A/C mains power supply and battery charger
803028	USB Drive, 32 Gb (Western Digital Sandisk # SDCZ60-032G-A46 - Factory tested for use with the EVOM3)
13142	Footswitch
3993	2 mm Adapter for EVOM2

ELECTRODES FOR TEER (EPITHELIAL) MEASUREMENT

STX2-PLUS	Replacement Electrode Set
STX2	Replacement Electrode Set (Requires 99672 for use with the EVOM3)
STX3	Adjustable Electrode Set for shallow wells, 5-9 mm depth (Requires 99672 for use with the EVOM3)
3993	2 mm Adapter for EVOM2

ENDOHH CHAMBERS FOR ENDOTHELIAL/EPITHELIAL MEASUREMENT

ENDOHH-6G	EndOhm for 6 mm culture cup (24 wells per plate)
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ENDOHM-12G	EndOhm for 12 mm culture cup (12 wells per plate)
ENDOHM-24G-SNAP	EndOhm for 24 mm and Costar Snapwell cup (6 wells per plate)
EVOM-CAL	EVOM STX Calibration Solution
TEER-CL	TEER Electrode Chloride Solution

EndOhm chambers require **EVOM3** cable **99916**.

Electrode Adapter (WPI# **3993**) converts into four independent 2 mm jack. It allows the user to utilize the **EVOM3** meter with another four-electrode system.

STX2-PLUS is the standard electrode for use with the **EVOM3** and is designed to facilitate measurements of membrane voltage and resistance (TEER) of cultured epithelia directly in tissue culture wells. The electrode incorporates a fixed pair of probes, 5 mm tall, 3 mm wide and 1 mm in thickness. Each probe has an inner (voltage) and an outer (current) electrode.

STX3 is an alternative electrode to the **STX2** that can be used with the **EVOM3** for shallow inserts (5–9 mm). It differs from the **STX2** in that the vertical distance between the probes of the **STX3** can be adjusted for up to an additional 10 mm of separation.

STX100C96 and **STX100M** are optional 96-well electrodes for use with the **EVOM3**. These electrodes have a smaller form factor specifically designed for high throughput screening (HTS) plates with higher well counts. The shape of the **STX100** electrode is keyed to the access ports of the well plate. This results in consistent spatial positioning leading to superior reproducibility from well to well.

NOTE: The **STX100C96** and **STX100M** require the **99672** EVOM2 electrode adaptor for use with the **EVOM3**.

Endohms are an optional electrode chambers for the **EVOM3** that may be used when working with well cups that are individually removable. It can also be used for studying the tight junction changes induced by chemicals and other factors. Concentric pairs of electrodes above and below the insert membrane results in excellent stability and reproducibility. (See "APPENDIX E: Improving the Accuracy and Repeatability of the System" on page 39.) Unlike the **STX2-PLUS**, the inserts must be transferred from the culture plate to the **Endohm** chamber to make a measurement.

TROUBLESHOOTING

EVOM3 Meter: Most of the time, system problems are related to the electrode, not the meter itself.

STX2 Electrodes: Some issues you may encounter include:

- **UNSTABLE OR HIGH READING:** If the electrode fails, the most common symptom is an unstable or unusually high reading.
- **LOW RESISTANCE:** When the meter displays a lower than expected resistance, but is stable and reproducible, the most likely cause is related to the cell culture, not necessarily the meter or the electrodes. Refer to Troubleshooting Chart "Resistance reading unusually low" on page 31 and the note on page 6. See "Testing the EVOM3 Meter (Resistance) and Calibration" on page 21 or "Testing the STX2-PLUS Electrodes" on page 28.

Issue	Possible Cause	Solution
Unit runs briefly, and powers off	Unit is insufficiently charged	Recharge the unit for at least 12 hours. NOTE: Make sure the EVOM3 is powered off when charging the battery.
Unit does not charge or charging LED not lit	Insecure connection at power input jack on meter	Verify that the power connector is securely connected at the meter power input jack.
	Defective charger	The EVOM3 charge socket can be tested with a USB to Mini-B cable plugged into a computer's USB port (WPI # 803026). If the charging LED lights up, then a new charger is needed (WPI # 803025).
USB drive not working or not recognized by EVOM3	Not all USB thumb drives work on the EVOM3.	See "Sample Resistance File (*.CSV)" on page 16 and page 5.
Meter will not power on with charger DISCONNECTED	Batteries are discharged	The meter powers down automatically when the batteries are depleted. Power off the meter and connect it to the battery charger. Charge it for at least 12 hours. A full charge may require up to 24 hours. For reliability, the meter should be charged each evening (with the power off) before use the next day.
	Batteries are defective	Contact WPI for service. The EVOM3 battery is not user servicable.
Voltage reading is unstable	Electrodes are heavily charged or dirty	Clean the electrodes. See "Cleaning/Maintaining the STX2-PLUS Electrodes" on page 26.
	Electrode too close to strong electromagnetic radiation device	Move the system to a different area away from sources of electromagnetic fields. Electromagnetic field sources could include computers, MRI equipment, magnetic stirrers, etc. Cell phone signals can also interfere.
	Power line or USB connected to a recording device causing noise	Remove the USB connection to eliminate the possibility of a ground loop.

Issue	Possible Cause	Solution
Voltage reading is not zero in plain culture media or blank cell	Electrode not equilibrated	The Ag/AgCl electrodes (inner voltage measurement electrodes) on the probe may become polarized due to an uneven distribution of ions between the electrode pair. This ionic charge can be neutralized by immersing the probe into 0.1M KCL for 24 hours with the probe electrodes shorted electrically. The probe is shorted electrically when it is connected to the meter with the power OFF, and the FUNCTION switch in the OHMS position. See "Probe Null" on page 14 for information on how to null the electrodes.
	Dirty electrode	Clean the electrode. (See "Cleaning/Maintaining the STX2-PLUS Electrodes" on page 26.)
	Contamination between electrodes	Inspect the inter-electrode surface areas for material which could form an insulative coating on the surface of the inner and outer electrodes. If the material cannot be removed, the electrode should be replaced. (See "Cleaning/Maintaining the STX2-PLUS Electrodes" on page 26.)
	Corrosion has formed on conductive traces	An insulative transparent coating protects the copper conductors that connect each electrode to the main wiring in the handle. When the probe is very old, this coating can deteriorate, allowing the salts in the media solution to leech through to the copper conductors. This is observed as a black discoloration on the copper circuit traces underneath the conformal coating. If this is observed, replace the electrode.
Resistance reading usually high	Contaminated electrode contacts	With use, the chopstick electrode probe contacts may acquire a buildup of protein or other foreign material that effectively increases the baseline resistance of the electrodes. The electrodes can be cleaned using various methods. (See "Cleaning/Maintaining the STX2-PLUS Electrodes" on page 26.)
Resistance reading unusually low	Cell culture or media problem	If the cell culture has been given sufficient time to achieve confluence, and the reading is stable but significantly lower than expected, then the problem is probably related to the cell culture. Electrode failure will not generally cause a lower than expected yet stable reading. Use the test resistor to verify the meter is functioning correctly. The meter display should read 1000Ω. See "Instrument Diagnostics and Calibration" on page 21, "Testing the STX2-PLUS Electrodes" on page 28 or refer to the appropriate electrode manual for instructions on testing the electrode. See "APPENDIX E: Improving the Accuracy and Repeatability of the System" on page 39.

Issue	Possible Cause	Solution
Resistance is a negative value	Contamination on electrodes	<p>Use the 1000Ω test resistor to verify that the meter is working correctly. The meter display should show 1000Ω. See "Instrument Diagnostics and Calibration" on page 21. If a negative value exists only when using the STX electrode, inspect and test the electrode for the possible formation of an insulative coating of foreign material that electrically blocks the current and voltage electrodes. Remove the foreign material and retest. See "Testing the STX2-PLUS Electrodes" on page 28 or refer to the appropriate electrode manual for instructions on testing the electrode. See "Appendix A: KCL Testing Mixtures" on page 35.</p> <p>If a red negative value shows on the display only when using the electrode and the blank setting is off or at zero, then inspect the electrode for the formation of an insulative coating of foreign material that electrically blocks the current and voltage electrodes.</p>
Resistance reading drifts or is unstable	Electrodes are not fully immersed in culture media solution	The meter will not provide a stable reading if the electrodes are disconnected from the meter or if the electrodes are not fully immersed into culture media.
	Electrodes are not held still during measurement	Handheld electrodes must be kept as motionless as possible during a measurement. Excessive movement will cause the measurement to fluctuate.
	Charger is connected to the meter	The meter reading can become unstable due to the loss of electrical isolation when the charger is connected to the AC power. To ensure stability of readings, always disconnect the charger from the meter when making measurements.
Resistance reading drifts or is unstable	Old electrode	Use the test resistor to verify the meter is functioning correctly. The meter display should read 1000 Ω. See "Instrument Diagnostics and Calibration" on page 21 or "Testing the STX2-PLUS Electrodes" on page 28. The lifetime of the electrodes is between 1- 2 years with normal use. See the previous note on corrosion and the note on coatings. See "Appendix A: KCL Testing Mixtures" on page 35.

Issue	Possible Cause	Solution
STX2-PLUS electrode tips over when released	The cable is heavy and can cause the electrode to tip over if too much 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 electrodes are coated with deposits from the use of DMEM.	The electrodes requires enzymatic cleaning.
	The fluid temperature in the plate is changing.	Use a plate warmer.
	In a 5% CO ₂ environment, a loss of CO ₂ causes the media pH to change, and the resistance reading may increase sharply.	A 5% CO ₂ environment can help in reducing pH media changes.
The electrode reading is unstable. (see the stability definition below).	The electrodes are coated.	The electrodes require enzymatic cleaning.
	The electrodes need to be chlorided.	The electrodes require chloriding. See "Rechloriding the Electrodes" on page 27.
	Radio frequency interference	Turn off or move any cellular phones farther away from the experimental setup.
---- appears on the screen	Resistance reading is out of range	An out of resistance range will display ---- on the screen. Adjust the range to correct the error. A saved well will read a space (" ") if the display is out of range. Go to Setup, Mode Units and increase the resistance range. See page 11. NOTE: The EVOM3 will read only about 5% above 100K Ω.

NOTE: If you have a problem/issue with that falls outside the definitions of this troubleshooting section, contact the WPI Technical Support team at 941.371.1003 or technicalsupport@wpiinc.com.

*Drift—Readings that continuously increase or decreases a significant value (either voltage or resistance) over time. Example 1: At 10 mV the PD reading is increasing 1 mV/minute. (A drift of 0.1 mV/minute is acceptable.) Example 2: At 1000 Ω the reading is increasing 100 Ω/minute. (A drift of 10 Ω/minute is acceptable.) Excessive drift may be caused by a coated electrode, pH or temperature changes. See the Troubleshooting entry on drifting above. Also see "Instrument Diagnostics and Calibration" on page 21 or "Testing the STX2-PLUS Electrodes" on page 28.

Instability—At 500 Ω, the reading jumps from 450 to 550 Ω and will 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 be coated with DMEM and require enzymatic cleaning. See "Cleaning/Maintaining the STX2-PLUS Electrodes" on page 26.

SPECIFICATIONS

This unit conforms to the following specifications:

Tissue Sampling Frequency	12.5 Hz
Sample Averaging	20 samples running average
Resistance Ranges.....	0 to 10,000 Ω
.....	0 to 50,000 Ω
.....	0 to 100,000 Ω +5%
Auto Mode	1 to 100,000 Ω auto current 2 μA, 4 μA, 10 μA
Resistance Resolution	0.1 Ω (under 200 Ω); 1 Ω (over 200 Ω)
Resistance Accuracy.....	0.1 Ω (under 200 Ω), 1 Ω (over 200 Ω) 0.1%
.....	100,000 Ω ± 2 μA (to 105 KΩ)
Voltage Resolution.....	0.001 V, 0.1 mV
Accuracy Resistance.....	0.1 Ω (200 Ω); 1 Ω (above 200 Ω)
Accuracy voltage	± 0.1 mV
Current Levels	10,000 Ω ±10 μA
.....	50,000 Ω ± 4 μA
.....	100,000 Ω ± 2 μA
.....	Auto mode 1 to 100,000 Ω auto current 2 μA, 4
Display Update Rate	0.5 seconds
Battery.....	3.7V Li-ion 2500 mAh**
Charging Period	5.5 hours (power off); 8 hours (run time)
Charge Current	200 mA
Power Consumption.....	~250 mA
Certifications.....	CE
Firmware.....	Upgradeable*

***NOTE:** A USB to Mini-B cable (WPI #803026) is required along with PC bootloader software and the image are required to upgrade the firmware.

**Not user serviceable. Contact WPI for repair or replacement.

APPENDIX A: KCL TESTING MIXTURES

When making a variety concentrations of KCl solution, use the following table as a guide. Start by making a solution of 160 mM concentration. To make 100 mL of 160 mM KCl:

Add 1.192 gm KCl to 100 mL of distilled water. (1M KCl = 74.54 gm/M in 1 L DI water)

Use	Add DIW	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
25 mL of 20 mM KCl	25 mL	50 mL 10 mM KCl

APPENDIX B: FILE FORMATS

Resistance (*.CSV)

Date 03/12/2020 13:11:56 Well Plate 24

EVOM3 Version 1.0

MODE OHMS offset 999.8

Units: OHMS

Direction 1

Resistance Scale 50k Ohms

0	1	2	3	4	5	6
A	19015	19015	19015	19015	19015	19015
B	19016	19016	19016	19016	19016	19016
C	19016	19016	19016	19016	19016	19014
D	19014	19014	19014	19014	19014	19017

Voltage (*.csv)

Date 03/12/2020 13:12:48 Well Plate 24

EVOM3 Version 1.0

MODE PD offset -0.0 mV

Units: mV

Direction 1

Resistance Scale 50k Ohms

0	1	2	3	4	5	6
A	0.0	0.0	0.0	0.0	0.0	0.0
B	0.0	0.0	0.0	0.0	0.0	0.0
C	0.0	0.0	0.0	0.0	0.0	0.0
D	0.0	0.0	0.0	0.0	0.0	-0.0

APPENDIX C: CONTINUOUS RECORDING VIA USB (PYTHON) ON A PC

The program on the USB thumb drive is a self-executing program to record serial data through a USB cable to a Window PC computer. This is intended to record continuous measurements of a single sample. The following files are included on the USB stick:

- CDM v2.12.28 WHQL Certified EVOM3 USB driver for Windows
- Python.exe Python recording program and other support folders for Windows
- Read_EVOM3_3.E.bat Batch file for executing the recording program
- WPI_EVOMCNX_3.E.py Python EVOM3 USB digital recording program
- WPI_EVOM3.csv Sample recording file

NOTE: An out of resistance range indication on the **EVOM3** screen will show -----. The Python output will list -99999.9. If you are using a lower range (10K or 50K), stop the output (X), increase the range and restart. The maximum resistance level that the **EVOM3** can measure is 10,5000 ohms.

Running the External Data Connection Output Program (PC, Mac, Linux)

Windows 8 or above users:

WPI provides a USB keydrive (also known as a thumb drive) with all the software needed to run the External Data Output program. To use these programs:

1. Connect the **EVOM3** to the USB port of the PC. The active remote connection port for the **EVOM3** service port is in the back of the unit as a USB-B connector [marked in red].



Fig. 50—Plug the USB-B (red) connector into the port on the back of the EVOM3.

The plug-and-play service from Microsoft should install the driver automatically, however if a driver is not found, there is a driver in the folder on the USB drive called CDM v2.12.28 WHQL Certified, which can be used install the driver.

2. Plug the USB thumb drive into a USB slot on your PC. If your PC does not have a spare USB slot, you will have use the Windows option [B] below.
3. Open a Windows Explorer window and navigate to the USB thumb drive folder on the PC. Run the bat file “WPI_EVOMCNX_3.E.bat.” The program will query the COM ports in use and inform you as to which port the **EVOM3** is connected.

4. Enter the filename for the Output File (ENTER for WPI_EVOM3.csv). Type in a filename and add the .csv extension or enter to accept the default.
5. Enter the time between readings in seconds. (Press **Enter** for minimum delay.) Enter a time of 0, 1, 2 or 5 seconds. (0 is fastest at about 0.3 seconds.) The program will run and update until ^X (control X) is typed. The readings are continuous at 12.5 Hz, always on. The time entered is the averaged reading taken at that time interval.
6. The default file name is WPI_EVOM3.csv. This file may be opened in Microsoft® Excel.

For Windows users that can not use a USB keydrive, or only have one USB port, or for Linux OS or Mac OS users

1. For Windows, Linux or Mac users that already have Python 3.8.0 or higher installed, skip to step 2. If you do not have Python installed, download and install Python for your operating system from www.python.org. Be sure to download the version appropriate for your operating system. The minimum supported Python version is Python 3.8.0
2. Make sure all the Python libraries are available as keyboard, serial and glob. From a command line window, run these commands (all lower case):
 pip install glob
 pip install serial
 pip install keyboard
3. Download the file **EVOM3** digital output archive for your operating system from <https://www.wpiinc.com/support/software-download/>. This archive has all the Python program files to use the external data output.
4. Extract the files to a WPI directory or a directory of your choice.
5. Connect the **EVOM3** to the USB port of the PC. The active remote connection port for the EVOM3 service port is in the back of the unit as a USB-B connector [marked in red].



Fig. 51—Plug the USB-B (red) connector into the port on the back of the EVOM3.

6. Open Python or a command window on your system.

7. At a command line or from python type this command : python WPI_EVOMCNX_3.E.py and press enter. If you are not running Python from the directory where WPI_EVOMCNX_3.E.py was extracted, you will have to change directory to that location.
8. Enter the filename for the Output File (ENTER for WPI_EVOM3.csv). Type in a filename and add the .csv extension or enter to accept the default.
9. Enter the time between readings in seconds. (ENTER for minimum delay.) Enter a time of 0, 1, 2 or 5 seconds. (0 is fastest at about 0.3 seconds.) The program will run and update until ^X (control X) is typed. The readings are continuous at 12.5 Hz, always on. The time entered is the averaged 20s/s reading taken at that time interval. Note: 2 seconds is recommended.
10. The default file name is WPI_EVOM3.csv. This file may be opened in Microsoft® Excel or any other spreadsheet program as a .CSV file

```
E:\>python WPI_EVOMCNX.py
#####
###          EVOM3CNX Version 3.E          ###
### World Precision Instruments, LLC ###
#####

Serial Ports Found ['COM1', 'COM3', 'COM5']

TESTING PORTS FOR EVOM3 ...

Found EVOM3 Port as COM5
Enter File Name for Output File (ENTER for WPI_EVOM3.csv):
Enter time between readings in sec (ENTER for minimum delay):1
*****

Opening WPI_EVOM3.csv for output...
Opening COM5 for data input
Press 'CTL-x' key to stop data collection
*****

### DATA COLLECTION STARTED ###
TIME: 12 Mar 2020 13:21:14  DATAPOINT: 0  VALUE: 19017.6
TIME: 12 Mar 2020 13:21:14  DATAPOINT: 1  VALUE: 19017.7
TIME: 12 Mar 2020 13:21:15  DATAPOINT: 2  VALUE: 19017.7
```

Sample CSV recording

```
TIME , DATA , VALUE
12 Mar 2020 13:24:35,0,0000.0
12 Mar 2020 13:24:36,1,19017.9
12 Mar 2020 13:24:36,2,19018.1
12 Mar 2020 13:24:36,3,19018.0
12 Mar 2020 13:24:37,4,19018.1
12 Mar 2020 13:24:37,5,19017.8
12 Mar 2020 13:24:37,6,19017.7
12 Mar 2020 13:24:37,7,19017.7
12 Mar 2020 13:24:38,8,19017.8
```

NOTE: Python is an open source programming language. Licensing information can be found on the Python website (<https://docs.python.org/3/license.html#terms-and-conditions-for-accessing-or-otherwise-using-python>.)

APPENDIX D: 24 MM DIAMETER (6-WELL) INSERTS

Note that the resistance readings from 24 mm diameter tissue culture inserts (6-well plates) obtained by using WPI's **STX** series of electrodes will be 1.8 to 2.2 times higher than that obtained using the **ENDOHM-24G**, because the **STX** electrode cannot deliver a uniform current density over the relatively large membrane through the small gap between the membrane and the bottom of the well. Therefore, the resistance reading of a 24 mm (6-well) diameter insert obtained by **STX** electrodes should not be used to calculate the unit area resistance. **STX** electrodes are only intended for providing a qualitative measurement of cell monolayer health and quantitative measurement of cell confluence. This is not a problem with smaller inserts, such as the 12 mm and 6 mm diameter inserts, because of the relatively smaller size of the membrane compared to the electrode. When the **EVOM3** is used with the **EndOhm**, it gives a repeatable and accurate value of membrane resistance consistent with that obtained by using a well-designed Ussing chamber. It is also consistent with that obtained by the **STX** electrode for the 12 mm and 6 mm diameter filter inserts.

NOTE: The unit area resistance is independent of the area of the membrane and may be used to compare data obtained from inserts of different sizes.

For 6-well plates (24 mm inserts), the **STX2-PLUS** can be used to take readings at the 12 o'clock, 3 o'clock, 6 o'clock and 9 o'clock positions to look for low resistance points in a 24 mm insert to test for confluence.

APPENDIX E: IMPROVING THE ACCURACY AND REPEATABILITY OF THE SYSTEM

Whenever possible, operate the **EVOM3** meter with the battery charger disconnected. This ensures maximum electrical isolation for the most accurate readings. When the charger remains connected to the meter during measurements, electrical noise can be introduced into the system. For the highest accuracy in measurement, WPI recommends disconnecting the unit from the AC power source.

For 24-well or 12-well measurements the **STX2-PLUS** electrode is capable of making precise measurements. The **STX2-PLUS** is designed primarily for 24-well inserts, and it can be used with 24-well HTS plates* and 12-well inserts* with good results as long as the placement is consistent. Consistent electrode placement and mechanical stability are essential for consistent resistance results. Try to keep the fluid levels and fluid temperatures constant. The use of a warming plate can add to measurement items and reduce CO₂ out gassing during readings. CO₂ out gassing can change the pH of the media and sharply increase a resistance reading. See "Tips and Advice" on page 6 and the notes on page 6.

*For 24-well HTS plates, keep the basal electrode centered in the basal access slot.

*For 12-well inserts, the **STX2-PLUS** will have to be hand held.

For measuring low-resistance tissue culture or for more precise measurement, you should consider using the EndOhm chamber, **STX2-PLUS** or the **STX100** electrodes instead of standard **STX2** or **STX3** electrodes. In some cases where the insert height is shorter than 9 mm the **STX3** must be used. The **99672** EVOM3 adaptor must be used for the **STX3**, **STX2**, **STX100** electrodes.

Although cells must be transferred from their culture wells to the EndOhm chamber for measurement, more accurate measurement of membrane resistance can be achieved. By introducing concentric pairs of electrodes above and beneath the membrane, the EndOhm reduces background resistance (with a blank cup inserted) from 150 Ω to less than 2 Ω . (**ENDOHM-6G** is higher). The shape of the current electrodes allows a more uniform current density to flow across the membrane. Fig. 52 demonstrates how the STX electrode measures TEER, and Fig. 53 shows how the EndOhm chamber measures TEER. With a fixed centered electrode geometry, the variation of readings on the same sample is 1-2 Ω , as compared to 5% of the total reading using the **STX2** electrodes. An EndOhm, together with the **EVOM3**, offers the most accurate, convenient and economical solution for trans membrane electrical resistance measurement. For automation of TEER measurements, consider the **REMS** AutoSampler that integrates a robotic system and computer interface to automatically measure tissue resistance in 24- and 96-well plates. The ability of the **REMS** AutoSampler to precisely locate the electrode results in highly reproducible TEER measurement.

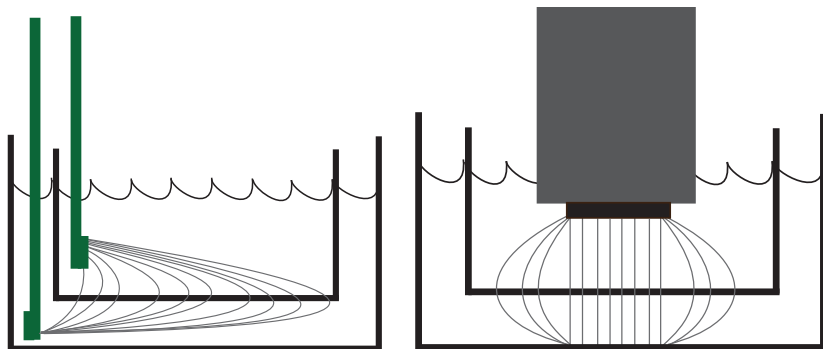


Fig. 52—(Left) The STX2 electrode measure TEER.

Fig. 53—(Right) The EndOhm electrode measure TEER.

APPENDIX F: RESISTANCE CALCULATIONS

Resistance

The value of the blank (control well) always adds to the total resistance measured across a tissue culture membrane. The blank resistance must be measured and then subtracted from the resistance reading across the tissue in order to obtain the true tissue resistance. For example, suppose the resistance through a 0.15 M KCl solution and across the membrane support (with no tissue present) of a 12-well cell culture insert measures 130 Ω . This is the blank or control reading for that cell culture insert. (Resistance may vary for culture cups made by other manufacturers.) In this example, using 800 Ω as the sample measurement, the calculated resistance for the tissue itself (R_{tissue}) is:

$$R_{\text{Total}} = 800 \ \Omega$$

$$R_{\text{blank}} = 130 \ \Omega$$

$$R_{\text{blank}} + R_{\text{true tissue}} = R_{\text{Total}}$$

$$R_{\text{true tissue}} = R_{\text{Total}} - R_{\text{blank}}$$

$$R_{\text{true tissue}} = 800 \ \Omega - 130 \ \Omega = 670 \ \Omega$$

Resistance Value of the “Blank” Insert

When using an **EVOM3** with an **STX2-PLUS** to measure a blank insert, the resistance value is typically between 60 to 180 Ω , depending on the specific brand of the insert, and lower in EndOhms. This is not background resistance due to the resistance of the blank filter. Rather, if the filter membrane is removed from the insert, the resistance reading of the insert will remain nearly the same, because the background resistance reading is due mainly to the electrode gap, fluid resistance, the small gap between the bottom of the cell culture insert and the bottom of the cell culture plate. See Fig. 54. For a Millipore insert this gap is about 1 mm, with some insert brands having a slightly larger gap than others. The variation in this gap is the cause of the difference between blank readings of different brands. The smaller the gap, the higher the electric resistance. The resistance of the filter membrane itself is actually negligible. If an **ENDOHM-24G-SNAP** or **ENDOHM-12G** chamber is used, the blank resistance becomes near zero, because the external electrode is directly underneath the filter and the gap does not exist.

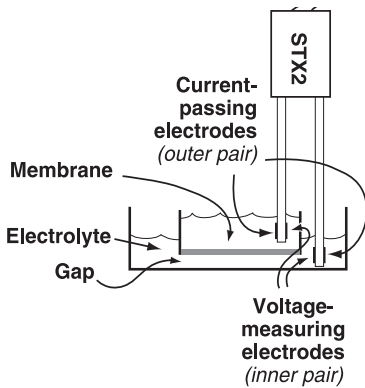


Fig. 54—Electrodes in solution

Unit Area Resistance

As the resistance is inversely proportional to the area of the tissue, instead of reporting resistance, typically the product of the resistance and the area is calculated and reported. The unit area resistance is independent of the area of the membrane used and may be used to compare data obtained from inserts of different sizes.

NOTE: Resistance readings for 24 mm or larger diameter inserts obtained by using the **EVOM3** with the STX electrodes should not be converted to unit area resistance. The EndOhm is recommended for these larger inserts. The unit area resistance is obtained by multiplying the meter readings by the effective surface area of the filter membrane. The dimension is $\Omega\text{-cm}^2$. The resistance is inversely proportional to the surface area. Thus, the larger the membrane, the lower the resistance.

Resistance of a unit area = Resistance (Ω) x Effective Membrane Area* (cm^2)

* See manufacturing specifications for the particular insert

Unit Area = 1 cm^2

The unit area resistance is independent of the area of the membrane used and may be used to compare data obtained from inserts of different sizes.

Continuing with the previous example, in which the $R_{\text{true tissue}} = 670 \Omega$, if an effective membrane diameter (d) was 6.5 mm, the unit area resistance would be:

Resistance x Effective Membrane Area = $670 \Omega * \pi r^2$

= $670 \Omega * 3.14 * 3.25 * 3.25 / 100$

= $670 \Omega * 0.331 \text{ cm}^2$

= $221.77 \Omega\text{-cm}^2$.

221.77 Ω is the resistance of a unit area of 1 cm^2 (TEER).

The larger the membrane, the lower the resistance. The dimension is $\Omega\text{-cm}^2$, not Ω / cm^2 .

TEER Calculations

TEER is a normalized value of resistance per 1 square centimeter of unit area. To compute TEER, multiply the measured resistance by the surface area's listed below.

For example, a 6.6 mm insert measures 1707 Ω , the TEER is 1707 Ω * 0.331 or 505 Ω .

- 6 well plate (24 mm inserts) 4.53 cm^2
- 12 well plate (12 mm inserts) 1.13 cm^2
- 24 well plate (6.5 mm inserts) 0.3316 cm^2
- 96 well plate (4.3 mm inserts) 0.145 cm^2

APPENDIX G: FREQUENTLY ASKED QUESTIONS

1. Will the **EVOM3** work with the EndOhms?

Answer: Yes, but an **99916** adaptor is required (or a new cable).

2. When do researchers want to use the blank function?

Answer: The blank function allows researchers to subtract out resistance measurement that is not caused by the membrane. This could be a reading affected by the electrodes or fluid resistances.

3. Does the system automatically calculate TEER?

Answer: No. Calculating TEER requires an area calculation. See "TEER Calculations" on page 2 and "Appendix F: Resistance Calculations" on page 41.)

4. The program is automatically stored when the last well is reached. What happens when I only want to measure 8 of 96 wells and store the program?

Answer: Once you're done measuring, open the settings, press the store screen menu, then store the plate data to the USB.

DECLARATION OF CONFORMITY



WORLD PRECISION INSTRUMENTS, LLC.

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E-mail: wpi@wpiinc.com

DECLARATION OF CONFORMITY CE

We: World Precision Instruments
175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA

As the **manufacturer/distributor** of the apparatus listed, declare under sole responsibility that the product(s):
WPI PN: EVOM3

To which this declaration relates is/are in conformity with the following standards or other normative documents:

Safety:
EN 61010-1:2010+A1:2019
EMC:
EN 61326-2-3:2013, EN 61326-1:2013
EN 61000-3-2:2014, EN 61000-3-3: 2013

And therefore conform(s) with the protection requirements of Council Directive 2014/30/EU relating to electromagnetic compatibility and Council Directive 2014/35/EU relating to safety requirements and Council Directive 2011/65/EU relating to hazardous substances:

Issued on: **March 31, 2020**

A handwritten signature in black ink, appearing to read 'Christopher C. Boldy', is written over a horizontal line.

Christopher C. Boldy
President of WPI



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 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.*

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