

INSTRUCTION MANUAL

EVM-MT-03-01 EVOM™ Manual for TEER Measurement

Serial No._____

www.wpiinc.com

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QUICK START

Resistance

- 1. Power on the **EVOM™ Manual** 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 **EVOM™ Manual** and begin measuring by pressing the foot switch or touching the *Store* button on the screen to store and advance to the next weel. 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. **EVOM™ Manual** 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 **EVOM™ Manual** 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 EVOM™ Manual 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. EVOM™ Manual 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 Ω * 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.

 $\underline{\mathbb{A}}$

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—EVOM™ Manual with a STX4 electrode.

INTRODUCTION

EVOM™ Manual is the WPI's newest instrument to measure Trans Epithelial Electrical Resistance (TEER) measurement. The **EVOM™ Manual** 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 **EVOM™ Manual** now has adjustable current levels in three fixed ranges with two lower ranges for sensitive membranes and high resistance ranges up to 100K Ω.

Confluence of a cellular monolayer is determined by an increase or a plateau in tissue resistance detected using the unique electronic circuit of the **EVOM™ Manual** and the **EVOM™ STX4** electrode. The **EVOM™ Manual** qualitatively measures cell monolayer health and quantitatively measures cell confluence. The **EVOM™ Manual** 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 **EVOM™ Manual** 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 **EVOM™ Manual** system makes this instrument ideal for permeability, PD and other detailed membrane studies.

EVOM™ Manual 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

EVOM™ Manual 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

Notes and Warnings

Choice of current/range: The **EVOM**^m **Manual** has three ranges of current from 2 μ A to 10 μ A and each current range has its own resistive upper limit up to 100K Ω :

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

The auto range will adjust the current as needed from 2 μ A to 10 μ A 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 **EVOM™ Manual**'s Li-ion battery will last 8 hours on its own. The charging time is 5.5 hours with the unit powered off. The **EVOM™ Manual** charger should not be plugged in all the time. The chager may be used to power the **EVOM™ Manual** 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 EVOM™ Manual.

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. **EVOM™ Manual** 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:

- Electrode resistance (Keep the electrodes clean.)
- 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 CO₂ 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 EVOM™ Manual 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 EVOM™ Manual will measure that as a lower resistance.

NOTE: Cleaning the electrode often corrects most of these issues. Refer to the Maintenance section of the electrode manual for cleaning protocols.

Charging the EVOM™ Manual

The **EVOM™ Manual** 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) EVOM™ Manual Epithelial Volt Ohm Meter

(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 31.)

- (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 **EVOM™ Manual** and **STX4** electrode can be downloaded at https://www.wpiinc.com/manuals.



Fig. 2—The EVOM™ Manual includes these parts.

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 39 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 39 of this manual.

INSTRUMENT DESCRIPTION EVOM™ Manual Meter



Fig. 3—USB port, Electrode connection



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

Theory of Operation

The **EVOM™ Manual** system uses an electrode assembly made of four subelectrodes, two for current sourcing and two for voltage measurement.

The **EVOM™ Manual** 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 µA) to minimize any tissue stimulation and to prevent the migration of metal ions.

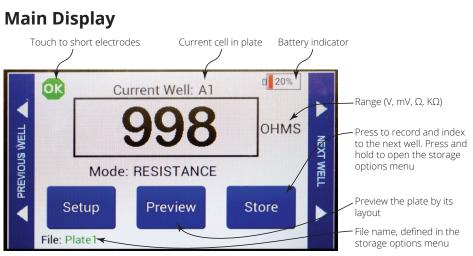


Fig. 5—The main EVOM™ Manual display.

A negative resistance number will appear in red text. This can be caused by a faulty electrode in media, (see "Troubleshooting" on page 25) or a blank setting that is over the measurement (see "Blank Handling Page" on page 12). When the meter is plugged into a power source, the battery icon shows a charging symbol.

Setup Page

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

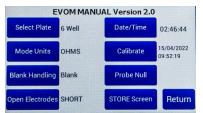


Fig. 6—Setup page

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 20.

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. 7—*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.
- 4. 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.

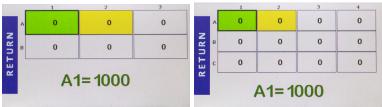


Fig. 8—(Left) 6-well plate preview Fig. 9—(Right) 12-well plate preview

| | | 1 | 2 | 3 | 4 | 5 | 6 | |
|-------|---|---|---|-----|------|---|---|-----|
| | A | 0 | 0 | .0 | 0 | 0 | 0 | |
| z | в | 0 | 0 | 0 | 0 | 0 | 0 | z |
| ¥ | с | 0 | 0 | 0 | 0 | 0 | 0 | ЦR |
| ETURN | D | 0 | 0 | 0 | 0 | 0 | 0 | E I |
| R | | | A | 1=1 | 1000 |) | | ~ |



Fig. 10—(Left) 24-well plate preview *Fig.* 11—(Right) 48-well plate preview

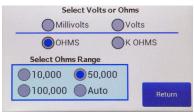
| | | 1 | 2 | 3 | 4 | 5 | 6 |
|--------|---|--------|---|------|------|---|----------|
| | A | 0 | 0 | 0 | 0 | 0 | 0 |
| | в | 0 | 0 | 0 | 0 | 0 | 0 |
| Z | c | 0 | 0 | 0 | 0 | 0 | 0 |
| RETURN | D | 0 | 0 | 0 | 0 | 0 | 0 |
| F | E | 0 | 0 | 0 | 0 | 0 | 0 |
| ш | F | 0 | 0 | 0 | 0 | 0 | 0 |
| | G | 0 | 0 | 0 | 0 | 0 | 0 |
| | н | 0 | 0 | 0 | 0 | 0 | 0 |
| | | < Scro | | 41 = | 1000 | | Scroll > |

Fig. 12—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

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



- Fig. 13—Choose the unit of measurement and (if you are using Ohms) the range.
- 2. 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 µA | | | | | | | |
| 50 K | 4 µA | | | | | | |
| 100 K | 2 µA | | | | | | |

| | Variable Current | | | | | | | |
|------|------------------|---------------|--|--|--|--|--|--|
| Auto | | 2 µA to 10 µA | | | | | | |

3. Press *Return*.

Blank Handling Page

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





- Fig. 14—(Left) Blank Handling window.
- Fig. 15—(Right) The main screen shows the offset underneath the reading.

- 2. Insert the probe into an empty well filled with media. Press *Reading* to make a measurement.
- 3. Select the *Blank Enable* radio button to enable the offset.
- 4. 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

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



Fig. 16—The Set Date/Time screen lets you set the date and time and choose the display format.

- 2. Press Set Date. Set the appropriate date and press Return.
- 3. Press *Date Format*. Choose your preferred format and press *Return*.



- Fig. 17—(Left) Set the date and press Return
- Fig. 18—(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. 19—(Left) Set the time and press Return Fig. 20—(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. 21—(Left) Null probe process.
- Fig. 22—(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. 23—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.



 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.

| Pla | te_ | | | | | | | | | | |
|-----|--------------------------|---|---|---|---|---|---|---|----|--------|--|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | Clear | |
| ۰a | b | С | d | е | f | g | h | i | j- | Cancel | |
| k | I | m | n | 0 | р | q | r | s | t | Gancer | |
| u | V | w | x | у | z | | • | • | 1 | Enter | |
| Сар | Caps off Space Backspace | | | | | | | | | | |

Fig. 24—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 **EVOM™ Manual** 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).
- 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 **EVOM™ Manual** 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
- EVOM™ Manual 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 EVOM™ Manual 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 |
| В | 19016 | 19016 | 19016 | 19016 | 19016 | 19016 |
| С | 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 EVOM™ Manual 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 |
| В | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| С | 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 **EVOM™ Manual**.
- 2. From the main page, press the *Settings* button. Press the *Store* button. Then, press the *USB Test* button.

| USB Memory st | ick Test |
|-----------------|------------|
| Press button to | start test |
| Test USB | Return |

Fig. 25—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.

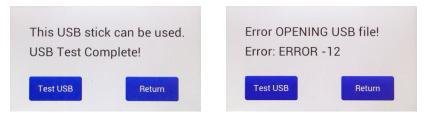


Fig. 26—(Left) The USB thumb drive passed the test and is suitable for use with the EVOM™ Manual.

Fig. 27—(Right) The USB thumb drive is not suitable for use with the EVOM™ Manual.

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. 28—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.



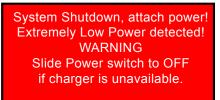
Fig. 29—The battery level is below 3%.

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. 30—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.



Shutdown in 60 seconds Extremely Low Power detected! WARNING Slide Power switch to OFF if charger is unavailable.

Fig. 31—When the battery power falls to 0%, a warning displays briefly. Fig. 32—The 60 second countdown was initiated when the battery power fell to 0%. Before shutdown, a final warning appears.

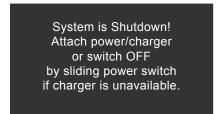


Fig. 33—Immediately before the system powers off, the shutdown message displays.

Reloading a Backup File after Power Failure

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



Fig. 34—(Left) Power level is restored when the unit is plugged into a power source.

 Cycle power on the EVOM™ Manual 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. 35—The backup file is restored on power up.

OPERATIONS

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

- Charge the **EVOM™ Manual** battery or run under mains power.
- Clean the electrodes. Refer to the Maintenance section of the electrode manual for cleaning protocols.
- If it is new, test the USB thumb drive. See "Sample Resistance File (*.CSV)" on page 16.
- Validate the EVOM™ Manual using 1000 Ω test resistor or calibrate the EVOM™ Manual.

Instrument Diagnostics and Calibration

WPI recommends that **EVOM™ Manual** 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 **EVOM™ Manual** operating status.

Testing the EVOM™ Manual 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 **EVOM™ Manual** reads 0 ±1 Ω.
- 6. To return to normal **EVOM™ Manual** operation, unplug the test resistor and press the **■** button (uppper left).

Testing the EVOM™ Manual 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*.
- 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 **EVOM™ Manual** 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. 36—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.

| Pla | te_ | | | | | | | | | |
|-----|----------------|---|---|-----|-----|----|---|-------|------|--------|
| 1 | 2 | 3 | 4 | 5 | 6 | 7. | 8 | 9 | 0 | Clear |
| ۰a | b | С | d | е | f | g | h | i | j- | Cancel |
| k | T | m | n | 0 | р | q | r | s | t | Gancer |
| u | ² v | w | x | у | z | , | | | ١ | Enter |
| Cap | os of | f | | Spa | ace | | | Backs | pace | |

- Fig. 37—Keyboard to enter a unique file name.
 - Press *Clear*.
 - Use the on screen keypad to enter a new name.
 - Press *Enter*.

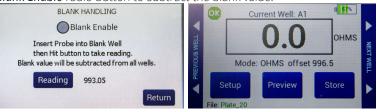
NOTE: Auto indexing changes the name to a numeric sequence formatted with the name, a prefix and a sequential number (name_prefixN).

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



Fig. 38—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 20.
- 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. 39—(Left) Blank Handling window.
- Fig. 40—(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 EVOM™ Manual 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. 41—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. EVOM™ Manual 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 **EVOM™ Manual** 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. 42—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

| Pla | te_ | | | | | | | | | |
|-----|------|---|---|-----|-----|---|---|-------|------|--------|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | Clear |
| ۰a | b | с | d | е | f | g | h | i | j_ | Cancel |
| k | | m | n | 0 | р | q | r | s | t | Cancel |
| u | v | w | x | у | z | - | • | | 1 | Enter |
| Сар | s of | f | | Spa | ace | | | Backs | pace | |

Fig. 43—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. 44—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. 45—(Left) Null probe process.

Fig. 46—*(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 EVOM™ Manual 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.

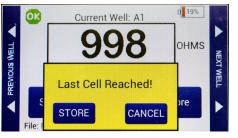


Fig. 47—Warning that the last cell measurement was taken.

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. EVOM™ Manual 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).

ACCESSORIES

| WPI Part # | Description |
|-----------------|---|
| EVM-EL-03-01-01 | EVOM™ EndOhm-6 Electrode for TEER in 6 mm Insert |
| EVM-EL-03-01-02 | EVOM™ EndOhm-12 Electrode for TEER in 12 mm Insert |
| EVM-EL-03-01-03 | EVOM™ EndOhm-24 Electrode for TEER in 24 mm Insert |
| EVM-EL-03-02-01 | EVOM™ STX HTS Electrode for TEER in Corning 96 |
| EVM-EL-03-02-02 | EVOM™ STX HTS Electrode for TEER in Milipore 96 |
| EVM-EL-03-02-03 | EVOM™ STX HTS Electrode for TEER in Corning Biocoat/Falcon 24 |
| EVM-EL-03-02-04 | EVOM™ STX HTS Electrode for TEER in Corning Costar 24 |
| EVM-EL-03-03-01 | EVOM™ STX4 Electrode with Removable Blades for TEER in 6 mm |
| | Inserts |
| EVM-AC-03-01-01 | EVOM™ Electrode Blades for the EVOM™ STX4 for TEER, 3 pairs |

TROUBLESHOOTING

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

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.

| 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 EVOM™ Manual is powered off when charging the battery. |
| b.0 | Insecure connection at power input jack on meter | Verify that the power connector is securely connected at the meter power input jack. |
| Unit does not charge or chargin LED not lit | Defective charger | The EVOM™ Manual 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 /not recognized | Not all USB thumb drives work on the EVOM™ Manual. | See "Sample Resistance File (*.ĆSV)" on page 16 and page 5. |

| Issue | Possible Cause | Solution |
|---|---|--|
| 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 EVOM™ Manual battery is not user servicable. |
| nstable | Electrodes are heavily charged or dirty | Clean the electrodes as per the instruction manual for the electrode. |
| Voltage reading is unstable | 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. |
| Voltage r | Power line or USB connected to a recording device causing noise | Remove the USB connection to eliminate the possibility of a ground loop. |
| Voltage reading is can not be set near zero | 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. |
| | Electrode requires cleaning | Clean the electrode as per the instructions manual for the electrode. |
| | 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. |
| | 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. |

| Issue | Possible Cause | | | | |
|--|--|--|--|--|--|
| Resistance reading unusually 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. | | | |
| Resistance reading unusually low | Cell culture or media problem \circ \circ \circ \circ \circ \circ \circ \circ \circ \circ | | | | |
| 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. | | | |
| Resista | Old electrode | Use the test resistor to verify the meter is functioning correctly. The meter display should read 1000 Ω . The lifetime of the electrodes is between 1- 2 years with normal use. See the previous note on corrosion and the note on coatings. | | | |

| Issue | Possible Cause | e Solution | | | | |
|---|---|---|--|--|--|--|
| Resistance is a negative value | Contamination on electrodes | working correctly. The meter display should show 1000Ω . 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. Refer to the appropriate electrode manual for instructions on testing the electrode. 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 reading appears to be drifting* (see the drift definition below). | The electrodes are coated with deposits from the use of media or buffer. | the current and voltage electrodes. The electrodes requires enzymatic cleaning. | | | | |
| | 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. | | | | |
| | 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 10. NOTE : The EVOM™ Manual 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 wpi-ts@wpiinc.com.

*Drift-Readings that continuously increase or decreases a significant value (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 unwanted deposits on the electrode, pH changes or temperature variance.

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 media or buffer constituents and may require enzymatic cleaning.

SPECIFICATIONS

This unit conforms to the following specifications:

| Tissue Campling Frequency | |
|-------------------------------------|---|
| | |
| | |
| | 0 to 10,000 Ω |
| | 0 to 50,000 Ω |
| | 0 to 100,000 Ω +5% |
| | 1 to 100,000 Ω auto current 2 μ A, 4 μ A, 10 μ A |
| | 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 | |
| | 0.1 Ω (200 Ω); 1 Ω (above 200 Ω) |
| 5 | ± 0.1 mV |
| | |
| | |
| | |
| | Auto mode 1 to 100,000 Ω auto current 2 μ A, 4 |
| | |
| Battery | |
| Charging Period | |
| 0 0 | |
| 0 | ~250 mA |
| | |
| | Upgradeable* |
| | 803026) is required along with PC bootloader |
| software and the image are required | |
| **Not user serviceable. Contact WPI | |
| ""NOT USEL SELVICEADIE. CONTACT WPT | or repair or replacement. |

APPENDIX A: FILE FORMATS

Resistance (*.CSV)

Date 03/12/2020 13:11:56 Well Plate 24 EVOM[™] Manual Version 2.0 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 |
| В | 19016 | 19016 | 19016 | 19016 | 19016 | 19016 |
| С | 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 EVOM[™] Manual Version 2.0 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 |
| В | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| С | 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 B: 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 EVOM™ Manual USB driver for Windows
- Python.exe Python recording program and other support folders for Windows
- Read_EVOM_HOMECNX.bat Batch file for executing the recording program
- WPI_EVOMCNX_5C.py Python EVOM™ Manual USB digital recording program
- WPI_EVOM_Manual.csv Sample recording file

NOTE: An out of resistance range indication on the **EVOM™ Manual** 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 **EVOM™ Manual** 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 **EVOM™ Manual** to the USB port of the PC. The active remote connection port for the **EVOM™ Manual** service port is in the back of the unit as a USB-B connector [marked in red].



Fig. 48—Plug the USB-B (red) connector into the port on the back of the EVOM™ Manual.

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 **EVOM™ Manual** is connected.

- 4. Enter the filename for the Output File (ENTER for WPI_EVOM[™] Manual.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 *Ente*r 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_EVOM[™] Manual.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 **EVOM™ Manual** 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 **EVOM™ Manual** to the USB port of the PC. The active remote connection port for the EVOM™ Manual service port is in the back of the unit as a USB-B connector [marked in red].

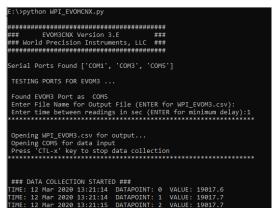


Fig. 49—Plug the USB-B (red) connector into the port on the back of the EVOM™ Manual.

- 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_EVOM_Manual.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_EVOM_Manual.csv. This file may be opened in Microsoft® Excel or any other spreadsheet program as a .CSV file



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 C: IMPROVING THE ACCURACY AND REPEATABILITY OF THE SYSTEM

Whenever possible, operate the **EVOM™ Manual** 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.

Choose an electrode based on your application (size of the inserts: 6 mm, 12 mm, 24 mm and transwell type).

- **EndOhm** electrodes by design are considered more accurate in capturing smaller changes. However, cell culture inserts need to be physically moved out of the plate to the **EndOhm** chamber for measurement.
- **STX HTS** electrodes are ideal for high throughput screening (HTS) plates.
- With STX electrodes, you can measure inside the well plate. The STX4 electrode is our new and improved version of the STX2-PLUS electrode. It has removable blades.

Fig. 50 demonstrates how the EVOMTM electrodes measures TEER. With a fixed centered electrode geometry of the **EndOhm**, 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 **EVOMTM Manual**, 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.

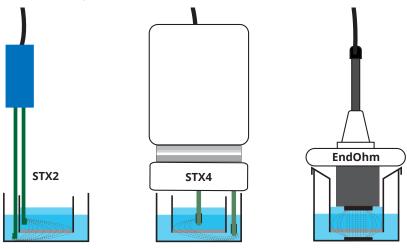


Fig. 50—This comparison shows how the electrode geometry can affect readings.

APPENDIX D: 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:

$$\begin{split} & \mathsf{R}_{\mathsf{Total}} = 800 \ \Omega \\ & \mathsf{R}_{\mathsf{blank}} = 130 \ \Omega \\ & \mathsf{R}_{\mathsf{blank}} + \mathsf{R}_{\mathsf{true \ tissue}} = \mathsf{R}_{\mathsf{Total}} \\ & \mathsf{R}_{\mathsf{true \ tissue}} = \mathsf{R}_{\mathsf{Total}} - \mathsf{R}_{\mathsf{blank}} \\ & \mathsf{R}_{\mathsf{true \ tissue}} = 800 \ \Omega - 130 \ \Omega = 670 \ \Omega \end{split}$$

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.

The unit area resistance is obtained by multiplying the meter readings by the effective surface area of the filter membrane. The dimension is Ω -cm². 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* (cm²)

* See manufacturing specifications for the particular insert

Unit Area = 1 cm²

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

Resistance x Effective Membrane Area = 670 Ω * π r²

= 670 Ω * 3.14* 3.25* 3.25 / 100

= 670 Ω * 0.331 cm²

= 221.77 Ω-cm².

221.77 Ω is the resistance of a unit area of 1 cm² (TEER).

The larger the membrane, the lower the resistance. The dimension is Ω -cm², not Ω / cm².

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 565 Ω .

- 6 well plate (24 mm inserts) 4.53 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

APPENDIX E: FREQUENTLY ASKED QUESTIONS

1. 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 cell layer. This could be a reading affected by the electrodes or fluid resistances or other factors. Normally, you would measure a blank insert with the same volumes as the insert and keep subtracting that blank value from the samples. This way, any offset contributed by the electrode is nullified. The subtracted value is the resistance contributed by the cellular layer.

3. Does the system automatically calculate TEER?

Answer: No. Calculating TEER requires an area calculation. See "TEER Calculations" on page 2 and "Appendix D: Resistance Calculations" on page 35.)

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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DECLARATION OF CONFORMITY CE

We:

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

As the **manufacture/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

President of WPI

F-QC-006

Rev C

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.

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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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