Applied BioPhysics
Quantifying Cell Behavior

TEER96 Manual

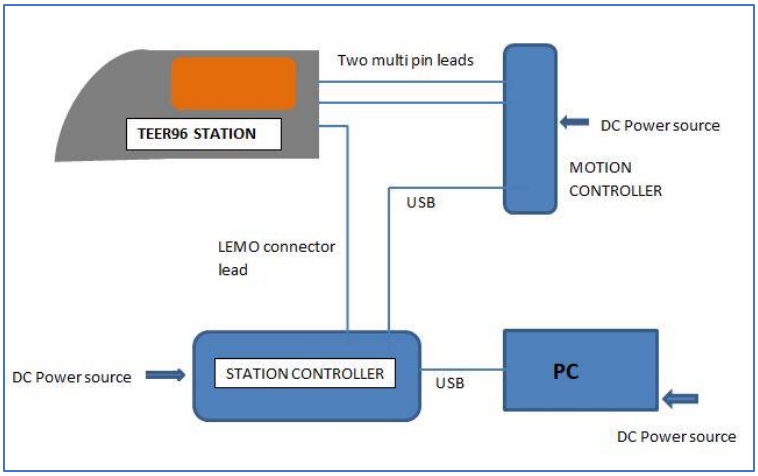
Contents

System Includes

- TEER96 Station
- Station Controller with power supply
- Motion Controller with power supply
- Laptop PC with TEER96 software installed
- TEER96 validation array
- Spare 96 dipping pin assembly
- Bar Code Scanner
- Bar code labels for culture filter plates
- 2 USB cables, LEMO cable, 2 Multi pin RS232 cable

System Setup

1) **Connect the components**
   a. Connect power cable to Station Controller and Motion Controller
   b. Connect laptop to Station Controller via USB cable.
   c. Connect Motion Controller to Station Controller via USB cable
   d. Connect Motion Controller to TEER96 Station with two 25 pin connectors
   e. Connect Station to Station Controller with LEMO lock-on connectors
   f. Connect power to PC; turn on PC
   g. Enter login with username: ECIS User (password not necessary)



2) **Placing the TEER96 Station in a high humidity incubator**
   a) If placing the TEER96 Station in the tissue culture incubator, to eliminate water condensation on the device, first warm the TEER96 in an airtight poly bag for 1-2 hour to allow equilibration to incubator temperatures. Once removed from the poly bag, connect the appropriate leads to the TEER96 Station.

3) **Start TEER96 software** Double-Click the TEER96 A icon and allow time to load.

Inserting the Dipping Pin Assembly

1) **Remove the bezel from the front of the TEER96 Station**
   a) Turn the two knurled machine screws on the front of the bezel and remove.
2) **Insert a sterile dipping pin assembly into the unit.**
   a) Slide the white Teflon rails into the grooves on either side of the opening and push lightly to assure the plug is seated in its socket.

3) **Replace the bezel* and hand-tighten the two knurled machine screws.**
   **It is important that the bezel always is mounted on the instrument when making filter measurements. The system motion control is calibrated to automatically halt loading a filter array with the lid in place. It is important that the correct filter plate, Millipore® or Corning®, be selected in the software drop down menu.*

Measurements with the TEER96 Instrument

How the TEER96 measurement works:

In TEER96 measurements, a confluent cell layer growing upon a filter is exposed to noninvasive 75 Hz AC electrical current, and the resulting voltage and phase information is used to determine the resistance of the cell layer. When this information is combined with the filter's area, the intrinsic barrier function of the cell layer is reported in ohm-cm^2.

To obtain the resistance of the cell layer, it is essential to account for all other sources of resistance and remove these from the final measurement. (*Much as in determining the weight of an object, one must first tare the balance to remove any source of weight except that of the material being measured.)*

The non-cellular sources of resistance involved in the TEER measurement include the series resistances associated with the two electrodes (Re), the solution resistance above and below the filter (Rs), and the filter itself (Rf).

R (without cells) = Re+Rs+Rf

After the cell layer has formed the series resistance due to the cell layer (Rcells) is added to this:

R (with cell layer) = Re+Rs+Rf+Rcells

The series resistance due only to the cell layer (Rcells) is calculated by subtraction.

To convert this series resistance into TEER values, the software calculates the equivalent resistance that would be measured from a parallel RC circuit where capacitance (C) is from the plasma membranes of the cells. This parallel resistance is then multiplied by the filter area to give the final TEER value.

Setting up a Zero Reference (cell-free) Required for All TEER Measurements

Note: The base filter plate for this, as well as all TEER measurements, must be the 96-well Transport Analysis Filter plate with individual wells and not the Single-well Feeder Filter plate. Also, before recording the zero values to be used, it is important that the medium is at the same temperature and pH as will be used in the cell-based TEER measurements.

The dipping pin assembly of the TEER96 is designed for use only with Millipore® Millicell® 96 cell culture insert plate (PSHT004R1 is 0.4um) *or Corning®* HTS transwell *96 tissue culture system (# 3381 is 0.4um).*

1) Zero Reference File (flat fielding)

a) Prepare a 96 filter plate assembly with all wells without cells. Use the same medium and volumes to be utilized in final TEER measurements (generally 235 and 75 microliters in the outer and inner well respectively).

The zero measurement will provide unique zero values for each of the 96 cell-free wells with their dipping pins, helping to eliminate any well-to-well variations in the actual TEER measurements. We refer to this recording of compensating open filter data as flat-fielding (a term from optical sensor data processing)

b) Select Eject to bring the platen out of the TEER96 instrument.

c) Place the filter plate with all wells having cell-free filters on the platen in the proper orientation. **Remove the lid** and select Load to move the filter plate into the device and upwards to receive the dipping pins. If you leave the lid on the plate the system will jamb and display a warning message to remove the lid.

d) Select Connect to confirm connections are in place. Upon completion of the measurement, all wells should be marked green. If for some reason contact is not properly achieved, the wells will be marked red. If this occurs, check well media volumes and check that the dipping pin assembly is firmly seated in the connecting socket.

e) Now click Zero and then Create Zero File.

f) Accept the name suggested by the software or provide another name for the file and click Save to begin the measurements. The newly recorded zero reference file will have the extension TEZ.

g) Upon completion of the measurement, the resistance value for each well will be displayed. Note: these values will later be used in calculating TEER values.

h) Click Eject to retrieve the filter plate from the instrument.

2) Select Cell-free Wells Method

a) Prepare a 96 filter plate assembly with at least one well without cells (additional cell-free wells are desirable). Use the same medium and volumes to be utilized in the cell containing wells (generally 235 and 75 microliters in the outer and inner well respectively for both Millipore and Corning).

b) In this procedure, the single well or group of wells are identified by the researcher as cell-free. The average values of these wells will be used in calculating the TEER values for other cell containing wells on the same filter plate. Unlike the flat-field method (see above), this method does not correct for well to well variations but will correct for any drift over time in the TEER values due to changes taking place in all wells e.g. incubator temperature changes.

c) Select Eject to bring the platen out of the TEER96 instrument.

d) Place the filter plate having some filters that are cell-free on the platen in the proper orientation. **Remove the lid** and select Load to move the filter plate into the device and upwards to receive the dipping pins.

e) Select Connect to confirm pins are in media and connections are in place. Use the toolbar selection tools if using a partial group of wells. All wells with media should be marked green. In the main display the resistance will be displayed.

f) Click Zero and then click Cell-Free Wells. The instrument will then read all wells and present the measured resistance for each well (the cell-free wells should show the lowest values). Next, select the cell-free wells by clicking on the desired wells in the lower left panel of the display – their centers will be marked white.

g) Click Start to begin the TEER measurements where the average values of the selected Cell-Free Wells will be used to calculate the TEER values of the other wells on the plate.

TEER Measurements of Cell Layers

The TEER96 operates in two modes of Measurements for either:

1) Single point in time measurement
2) Continuous time course measurement

1) Setup for Single Point Measurement of Multiple Plates

a) Go to the Acquire dropdown menu and check Single Point Read

b) Next, go to Read Options and set up the various parameters listed below:

- **Average Data Points** Set the Number of points to be averaged in measuring each well; default is one (1). Note: this choice will determine the length of time to measure the filter plate, one being the shortest time.

- **Use Same Zero File** - if this feature is set to **On** (default), the zero file selected for the first filter plate will be used for subsequent filter plates. [Please note, a zero reference file (cell-free filters) is required for all TEER measurements]. If **Off**, a new zero file will be requested for each plate.

- **Auto Cyle Read**– if **On**, the motion system will automatically request the next filter plate for insertion and will continue with additional filter plates until Finish is chosen.

- **Auto Start** (can also be used for robotic plate insertion) Filter plate sensor directed **Start** - if **On**, the filter plate lighted sensor on the back lefthand side of the platen is enabled. When a filter plate is placed on the platen, it is detected, and the system will pause X seconds (set in Auto Start Delay) before automatically loading the filter plate and running a read cycle. If using this feature, be certain to **remove the lid for each filter plate either before or immediately after placement upon the platen. If you leave the lid on, the system will jamb and a warning message will ask to remove the lid.**

- **Auto Start Delay** (sec) The amount of time the system waits after detecting a plate before automatically loading the plate

- **# Wash Plates** (max 3) This specifies how many different wash plates will be used in a wash cycle.

- **Wash dwell time** (sec) Amount of time in seconds the dipping pins remain in wash bath; default is one sec.

- **Wash Up/Down Cycles** This specifies how many times the wash bath is moving up and down on the dipping pins

  Wash Name 1 Enter the liquid used in this wash bath (water, alcohol, rinse, etc)

  Wash Name 2 Enter the liquid used in this wash bath (water, alcohol, rinse, etc)

  Wash Name 3 Enter the liquid used in this wash bath (water, alcohol, rinse, etc)

- **# Plate Reads between Washes** The number of plates to be measured before a wash cycle is started; default is one where a wash is initiated between every plate read. If the overall run is terminated before all the desired plates are run (by choosing Finish), the plate counter will be reset to zero.

NOTE: **WASH X** A wash cycle can be manually initiated from the main screen control panel. The X is a count down on the number of plates which have been read between wash cycles.

Click OK to save settings.

Starting Reading of Multiple Plates

a) Select Eject to bring the platen out of the TEER96 instrument.

b) Place the first 96 well filter plate on the platen in the proper orientation.

c) **Remove the lid** and Select Load to move the filter plate into the device and upwards to receive the dipping pins.

d) Click on Connect and then Zero choosing Load Zero File. Open the proper zero file (TEZ extension) that matches the dipping pin set loaded in the instrument.

e) Select Read, upon completion, the measured values will be displayed and color coded based upon the thresholds entered in the Single Point Measure Options menu. The data is stored in the named file that will have a TER extension. Following this, the plate will automatically be ejected, and the platen is ready for the next plate to be loaded.

   **We recommend scanning the serial number of the filter plate as this number will become a part of the file name*

f) Load the second plate and select Read and you will again be prompted to scan (enter) the serial number of the filter plate or input another file name. Upon clicking OK, the instrument will load and read the plate. Continue loading and reading additional filter plates as specified in the setup menu items.

g) To end the series of readings, Cancel the "Load Next Array" request, and then click Finish.

Quick Read feature

This option is used to provide a rapid estimate of the TEER values of each selected well. In this mode, a factory default cell-free value (600 ohms) is used for all wells when calculating TEER and wells will be colored using the Min/Max defined in Read Options. The TEER values of the quick read can be changed under Acquire, TEER Parameters

1) Load plate
2) Click Connect to confirm connections
3) Click Quick Read

2) Continuous Time Course Measurements

a) In the Acquire drop down, turn off Single Point Read option (uncheck)

b) Select Eject to bring the platen out of the TEER96

c) Place the 96 well filter plate on the platen in the proper orientation

d) **Remove the lid** and select Load to move the filter plate into the device and upwards to receive the dipping pins.

e) Select Connect to confirm pins are in media and connections are made.

f) Select Zero and then Create Zero or Load Dataset (use the TEZ file that matches the dipping pin set being used). The resistance values for each well of the TEZ file will be displayed. (see previous section on zeroing).

Alternatively, Select Cell-free Wells when cell-free wells have been included on the plate to be measured. The instrument will then read all wells and present the measured resistance for each well (the cell-free wells should show the lowest values). Next, select the cell-free wells by clicking on the desired wells in the lower left panel of the display – their centers will be marked white.

g) Select Start and name the file where data will be stored. Upon the Save command, the instrument will begin taking and storing data.

h) Press Pause during an experiment for cell treatment, etc. If the filter plate is removed from the Station, once re-inserted press Check to make sure all the wells are being read properly (TEER values will be displayed), then select Resume to continue the experiment.

i) To end the experiment press Finish.

Validating the Electronics:

1) Remove the orange bezel from the front panel of the TEER96 Station by unscrewing the two knurled machine screws.
2) Pull on the white Teflon frame to remove the dipping pin assembly.
3) Locate the validation assembly included with the system. This consist of an array of resistance values corresponding to different TEER values that were measured at the factory and the data stored in your instrument.
4) Insert the validation array into the opening such that the 100 pin connector on the PCB card is firmly seated into the socket at the back of the Station.
5) Click on Connect (the instrument will indicate **no array** is loaded. Click OK, then click OK again. This will override the platen array detect sensor) and when completed, click on the Validate Assay in the Acquire dropdown menu. Enter the Validation Array Serial Number found on the top of the array and click OK.
6) The instrument will automatically measure the validation array and display the card's 96 positions, lighting up green if all values are within 2% of factory measured values.
7) Should any position light up red, repeat the above steps checking that the validation array is seated correctly. Should any problems persist, please contact Applied BioPhysics Customer Service (www.biophysics.com; (518) 880-6860).
8) Remove the validation array, replace the dipping pin assembly, and replace the bezel on the front panel.

Cleaning and Replacing the Dipping Pin Assembly:

Although there is a provision to insert different wash baths and use the stepper motors to clean the dipping pins between filter plate readings, a more thorough cleaning and sterilization are recommended between different experiments or at the end of a series of multiple filter plate measurements. This, of course, is at the discretion of the researcher. Below are suggested steps.

1) Remove the dipping pin assembly
    a) Remove the orange bezel with its shield, to cover the electronics, from the front panel of the TEER96 Station by unscrewing the two knurled machine screws.
2) Pull on the white Teflon frame to disconnect and remove the dipping pin assembly.Clean the pins
    a) Soak the dipping pins in a tissue culture compatible detergent bath at 50^0 C or higher for 1 hr.
    b) Thoroughly rinse the device under running tap water
    c) Continue rinsing with distilled water being certain to remove all traces of detergent and tap water.
3) Sterilization
    a) Wrap the device completely with aluminum foil
    b) Sterilization can be accomplished by standard autoclaving or using alcohol with thorough rinsing and drying. **Do not sterilize using a high-temperature oven**.

Menu bar commands

File

- Open — Loads a previous experiment to allow new data to be appended to it
- Recent files — Loads a recent experiment
- Export Graph — Exports the current graph in a figure format (jpg, tif png, …)
- Export TEER — Exports the current experiment to a csv file
- Close — Finishes data acquisition
- Exit — End the program

Edit

- Copy Graph — Copies the graph in a PDF
- Export Graph — Exports the current graph ina figure format (jpg, tif, png,..)
- Color Palette — Allows editing of well colors
- Error Bars — Selects error bars as SD or SEM

Acquire

- Single Point Read — Turns on single point measurement mode (Off for continuous time course measurement)
- Read Options- sets up loading/unloading of plates, wash cycles, etc
- Home/Park Holder - Moves platen to a park position within the TEER96 station (not raised to incorporate dipping pins). This step also recalibrates the motors controlling the movement of the platen
- Setup new experiment - Ctrl+S Same as pushing Connect on the main screen
- Activate all wells- turns on all wells
- Find Instrument - Set ECIS COM port (found in Windows device manager) and connects to instrument, if asked start with "1"
- TEER Parameters:
    - Filter Surface Area - for Millipore filter plate is 0.143 cm^2 , Corning Plate is 0.143 cm^2
    - Capacitance (used in TEER calculations) set for 0.66 microFarads/cm2

- Quick Read Reference Resistance - Open well resistance in ohms – set for 500 ohms
- Low, medium, target, high TEER -sets color threshold on color palette

- Use Barcode: on/off; includes bar code plate number in the data and zero file

- Validate Assay - Requires installation of the validation test board (included with the system). This board has a series of known resistors used in calculating TEER values for each well position and is used to test the system electronics. If within 2%, will be green.
- Zero/cell Free Ref:

  Set Cell Free Wells- you will also be prompted to select the cell free reference wells when setting up a run

  Clear Cell Free Wells - clears choosen cell free wells
  Display Zero Ref. - displays the cell free well reference data file being used

- Plot Data Rate sets data rate to read plate at 1, 5, 15 or 60 minute intervals
- Plate type - Millipore® or Corning®

Help

- Manual - Opens PDF version of manual
- Open Log File - Opens the serial log file for inspection and troubleshooting. Saves in notepad after a run is complete and sent to the factory
- About - lists software version

Comments, Data Manager, Close

Comments:

- Includes data file, date, time , frequency, filter area, wells selected list of Restance and TEER values, pause and mark information is recorded in comments
- Customer can write any information they desire into the comments section. This is included with the data file

Data Manager:

- Allows the ability to open up previous data files while acquiring data.

Close:

Closes an open data file

File structure:

Single point read mode: Bar code under Aquire turned **off**

- Data file single point read:
  - TEER96_A03903_ID_20191210T154314_READ01.TER
    Instrument model, instrument serial number, year, month, day, hour, min, sec, single point read sequence.TEER file extension

- Zero file:
  - TEER96_A03003_191210_SFT_ZERO_2.TEZ
    Instrument model, instrument serial number, year, month, day, Single Frequency TEER, read sequence.TEER file extension

Data File single point read: Bar code under Acquire turned **on**

- Data File Single point read:
  - TEER96_A03003_191210_ID_1234_READ19.TER
    Instrument model, instrument serial number, bar code number, single point read sequence. TEER file extension

- Zero file:
  - TEER96_A03903_191210_ID_1234_SFT_zero_1. TEZ
    Instrument model, instrument serial number, year, month, day, bar code number, Single frequency TEER, zero file, read sequence. TEER file extension

Continuous time course measurement mode;

- Data File countinoues time course: Bar code under Acquire turned **off**

  - TEER96-A03903-191210_ SFT_EXP_02.TER
    Instrument model, instrument serial number,year, month, day, Single Frequency TEER, continuous measure experiment sequence number. TEER File extension

- Zero File:
  - TEER96-A03093-191210_SFT_Zero_1.TEZ
    Instrument model, instrument serial number,year, month, day, Single Frequency Timecourse, sequence number. TEER Zero file extension

- Data File countinoues time course:Bar code under Acquire turned **on**

  - TEER96-A03903-191210_ID_X0001_SFT_EXP_02.TER
    Instrument model, instrument serial number, bar code number, Single Frequency TEER, continuous measure experiment sequence number. TEER file extension

- Zero File:
    - TEER96_A03903_192110_ID_X0001_SFT_ZERO_1.TEZ
      Instrument model, instrument serial number,year, month, day, bar code number, Single Frequency TEER, sequence number. Zero file extension

600.032R2	Requires version V2.0.54.7 or higher