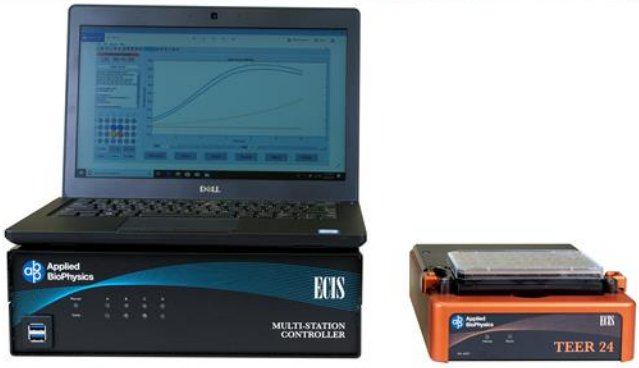
Applied Biophysics
Quantifying Cell Behavior

TEER24 MANUAL



The ECIS TEER24 is designed to carry out traditional trans-epithelial/endothelial electrical resistance measurements using ECIS (electric cell-substrate impedance sensing) technology to monitor the barrier function of cell layers grown on membrane insert filters. Data is collected continuously from up to 24 independent wells and reported as real-time barrier function changes in ohm-cm^2.

Contents:

Setting up the TEER24

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System includes:
- TEER24 Station
- Station Controller with power supply
- Laptop PC with TEER24 software installed
- 6 24 well TEER24 microplates with lids
- 1 Common Electrode Array with 24 gold pins (CEA)
- TEER24 Validation Array
- 1 USB cables
- 1 LEMO Cable

Station Controller

Station

LEMO Cable

USB Cable

Power Cable

TEER24 Microplate

Common Electrode Array

Validation Array

1) System Setup

a) Remove components from packaging

b) Connect power cable to Station Controller and into wall outlet (If there is concern about quality of power, an Uninterruptible Power Supply (UPS) is recommended).

c) Connect laptop to Station Controller by running USB cable from laptop to the Computer USB port on Station Controller.

d) Connect Station Controller to TEER24 Station by running the LEMO cable from **Unit A** port on back of Station Controller to LEMO port on back of TEER24 Station.

2) Mount Validation Array to TEER24 Station

a) On TEER24 Station, slide the two retaining clips towards outside of station.

b) Insert Validation Array into Station using correct orientation and favoring the upper-left corner.

c) Push down on one side of Validation Array and slide retaining clip inward to hold plate down; repeat on opposite side.

3) Start TEER24 Software

a) Double-click TEER24 Icon and allow load time.

b) Press **Connect** to allow software to recognize attached wells.

c) All 24 wells should appear green in the Well Map. If any wells appear red, reseat Validation Array and re-press **Connect**.

d) Repeat until all wells appear green in Well Map

4) Validate Array

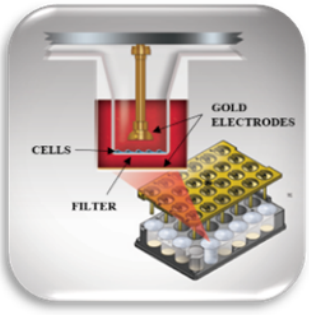
a) Select "Acquire → Validate Assay" from Menu bar.

b) Enter serial number of Validation Array when prompted.

c) Values of the resistance and TEER will be shown. These are compared to measured values from the Validation Array when the instrument was setup and tested at the factory.

d) Wells will appear...
- Green if values are within 5% of expected values
- Red if values are over 5% of expected values

*If wells appear red, try remounting and connecting validation array or contact customer service.



Running a TEER24 Experiment

Recommended Protocol for Following the Development of TEER in Freshly Inoculated Filters

The following protocol is recommended for time-course changes in the values of TEER following the inoculation of filter membrane inserts. This protocol assumes the TEER24 Station is within the incubator space and fully warmed to incubator temperature and that the common electrode array (CEA) or dipping pin assembly has been thoroughly cleaned and sterilized.

1. Equilibration of the TEER Assembly

a. Fill each well of the base plate to be used with 1.5 ml of culture medium.
b. Place the common electrode array (CEA) in the wells and put the lid in place.
c. Place the complete TEER assembly on a shelf within the incubator (there is no need at this time to clamp the unit in the TEER24 Station)
d. Allow the dipping pins to soak in medium and the system to reach thermal equilibrium for at least one hour.

2. Preparation of Cell Suspension

a. Pre-warm medium to 37C
b. Prepare cell suspensions with the required number of cells. 200 ul of cell suspension will ultimately be added to the inner well to attach to the filter substrate area.

For Corning membrane inserts the filter area is 0.33 cm^2. As an example, if one wishes to have a final cell density of 10^5 cells per cm^2, then 200 microliters of a 16.5 x 10^4 cells/ml suspension should be used.

3. Addition of Filters to the Microplate and Inoculation

a. Remove the dipping pins (CEA) and set it aside on a sterile surface.
b. Change the volume in the base plate wells to 1.0 ml of medium (either replace the equilibration media or remove 0.5 ml from each well)
c. Add the membrane inserts and fill each inner well with 200 microliters of cell suspension or medium only (at least one and preferably two wells should be cell-free).
d. Insert the CEA into the baseplate and cover with the lid

4. Mounting the Inoculated Array Assembly in the TEER24 Station

a. Insert the complete well assembly on the pogo pin bed
b. Move plate to the upper left corner
c. **Being careful to not apply pressure at any time to the central region of the lid**, push down on the right slide of the plate, and, lifting the lid slightly, slide the retaining clip inward.
d. Repeat this on the left side. **Again, do not push down on the central region of the lid,** as this may damage the mylar electrode film.
e. If the Station was removed from the incubator to carry out the mounting, return the device with the mounted array to the incubator

5. Running the ECIS TEER24 Software

a) Double-click the TEER24 icon to open software
b) Press **Connect** to test connection of each well (shown in the Well Map)
   i. All wells with medium display green; well without medium or not connected will display red
   ii. If necessary, reseat the well assembly and retest connections
c) Allow time for the array to equilibrate to incubator conditions

Note: It is best to wait as long as possible. As barrier function usually requires several hours to become established, one can consider waiting an hour or more before continuing with the zeroing process.

d) Once the equilibration is completed, press **Zero** to set the zero reference. A popup window will allow three options: choose to **Create Zero File**
e) A diagram of the wells and the measured resistance values will be displayed
f) Click on the cell-free wells in the Well Map (their centers will display white)
g) Click **Start** to begin the time course measurement
h) During the experiment, **Pause** may be pressed to stop data collection temporarily during cell treatment, etc. Note: the clock recording experimental time will continue running

When placing the well assembly back into the TEER24 Station, follow Step 4 above. Once mounted, press **Check** to confirm connections. The current TEER values will be displayed in the graphed circles. Press **Resume** to continue the experiment

i) To end the experiment, press **Finish**

Running a TEER24 Experiment

Recommended Protocols to Follow TEER Upon Addition of Drugs and Other Bioactive Materials

1. If one is already following the development of TEER (see above protocol):

a) **Pause** the measurement and remove the array assembly to a sterile hood.

b) Remove the lid and dipping pin assembly and make any required media changes in the wells (e.g., serum free medium) using prewarmed solutions. These changes should include the cell-free wells as well as the experimental wells.

c) Return the system to the TEER24 Station and when clamped in place, click **Check** to assure connections are re-established.

d) Next click **Resume** and continue to follow time course changes

e) When alterations in TEER due to the addition of different media have stabilized the system is ready for drug addition.

f) **Pause** the measurement and in the hood and using prewarmed solutions add drug dilutions to the experimental wells. Be sure to treat the cell-free wells identically but generally the drug need not be included.

g) Return the system to the incubator, click **Check** and if connections are re-established, click **Resume** to continue the time course to observe the effect of the drug.

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2. If one has inoculated filters but has not followed the development of TEER

a) Place the filters (including cell-free control filters) in the black base plate having 1 ml of medium in the large outer well and 200 microliters in the inner well.

b) Insert a prewarmed set of dipping electrodes (CEA)

c) Place the complete well assembly in the TEER24 Station

I. Insert the complete well assembly on the pogo pin bed

II. Move plate to the upper left corner

III. **Being careful to not apply pressure at any time to the central region of the lid**, push down on the right slide of the plate, and, lifting the lid slightly, slide the retaining clip inward.

IV. Repeat this on the left side. **Again, do not push down on the central region of the lid,** as this may damage the mylar electrode film.

V. If the Station was removed from the incubator to carry out the mounting, return the device with the mounted array to the incubator

d) Press **Connect** to determine if contact is established.

e) Wait an hour or more for equilibration of the electrodes before continuing

f) Press **Zero** and then **Cell-free Wells**

g) After the system reports the resistance of each well you will be asked to identify the cell-free wells (essential for this measurement) by clicking on the appropriate wells in the lower left display.

h) Click **Start** to begin the time course TEER measurement

i) Follow steps in the previous protocol (1-7) for drug additions

Setting up a Cell-free Zero Reference (Required)

Three Methods of Zeroing

1. Create Zero File

In most cases this approach is recommended as this zero measurement will provide unique values for each of the cell-free wells, helping to eliminate any well-to-well variations in the final measurements. These variations are mostly due to subtle differences in the electrode resistances that depend primarily on the history of the electrodes and their cleaning. In this flat fielding method, each well is measured without cells or with cells but before the establishment of a measurable barrier function (see suggested protocol). These values are applied in the final TEER calculations.*

**This process of recording compensating cell-free filter data for each well is referred to as flat-fielding (a term taken from optical sensor data processing)*

Procedure:

a) Prepare a 24 well filter assembly with the same medium and volumes to be utilized in final TEER measurements (generally 1.0 ml and 200 microliters in the outer and inner well, respectively).

b) Clamp the plate in place with the upper left corner (A1 well) pushed into the corner of the array holder to provide a proper connection with the pogo pins. **Be certain not to apply force to the central region of the plate.**

c) Select **Connect** to confirm connections are in place. Upon completion of the measurement, all wells with medium 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 be certain the plate is positioned as described above.

d) Allow sufficient time (an hour or more) to assure the medium is fully equilibrated with incubator conditions of temperature and pH, and then click Zero and then **Create Zero File**.

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

f) Upon completion of the zero measurement, the resistance value for each well will be displayed and stored in memory and you will be asked to identify cell-free wells. These wells will correct for any changes in the non-cellular resistance due to changes in incubator conditions (i.e. temperature changes) while the experiment is running.

g) If **Start** is then selected, these zero values will be the ones used in calculating the displayed TEER values.

*Note: Any TEZ zero file can be called up for later use by clicking **Load Zero File** and selecting the appropriate file. The resistance values of the previously measured zero will be displayed, and these values will then be used when **Start** is selected. Be aware that drifts in the cell-free electrode values from the previous zero run and the current time course run will be displayed as part of the TEER values.*

Setting up a Cell-free Zero Reference (Required)

2. Cell-free Wells

This approach is generally useful for cell layers having relatively high TEER values (>50 ohm cm^2) where subtle differences in cell-free well values are not an important factor. In this procedure, there must be at least one well that is cell free (additional wells are recommended). The remaining wells can be inoculated with cells or even have complete cell layers in place. Unlike the flat-field method (see above) where each well uses its own zero reference, this method applies only selected cell-free wells' zero reference to all of the wells. This method does not correct for well-to-well variations but will correct for any drift in the TEER values due to changes taking place in all wells, e.g. electrode conditioning or incubator temperature changes.

Procedure:

a) Prepare a filter plate with inoculated filters having at least one well without cells. Use the same medium and volume in the cell-free wells as in the cell containing wells (generally 1.0 ml and 200 microliters in the outer and inner well, respectively). It is essential to have all media at incubation temperatures and pH, or changes in reported TEER values, unrelated to the cells, may be observed.

b) In this procedure, the single well or group of wells will be identified by the researcher as cell-free. The average values of these wells will be used in calculating the TEER values for the cell-containing wells.

c) Clamp the plate in place as described above and 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

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

e) Click **Start** to begin time-course measurements.

3. Zeroing for Quick Read

The Quick Read option is used to provide a rapid estimate of the TEER values of each selected well using a fixed cell-free value. This approach will not provide a time course, but rather a single measure of the current TEER value of each well.

Procedure:

a) Clamp the plate in place as described above and 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

b) Select the fixed cell-free value to be used (the default is 250 ohms). Click the **Acquire** drop-down menu and choose **Teer Parameters**, then select **Reference Resistance** and change type in the cell-free resistance to the value to be used in calculating *TEER*.

c) Click the **Quick Read** button. The system will read all wells and present in a display of the wells the actual resistance measured and the calculated TEER (ohm-cm^2)

Maintenance of the Array Assembly

Cleaning the Common Electrode Array

To prevent unwanted drift and to improve the accuracy and repeatability of the measurements, it is essential to clean the dipping pin Common Electrode Array (CEA)

Cleaning:

a) Soak the dipping pin assembly in a hot detergent solution (e.g., 1%Liquinox® @ 70C) for 30 minutes or more.

b) Thoroughly rinse the device under running tap water to remove all traces of detergent

c) Continue rinsing with distilled water being sure to remove all traces of tap water.

Sterilization:

a) Sterilize by autoclaving or a high-temperature oven.

b) 70% isopropanol can also be effective

Cleaning the TEER24 Microplate array:

This consumable array is shipped sterile and, properly cared for can be used for a few assays before being replaced. To reuse the array follow the cleaning steps below:

a) After use, thoroughly rinse medium from the well with running water and allow it to soak in a warm detergent solutions for 30 minutes or more (1% Liquinox® @40C)

b) Thoroughly rinse the device under running tap water to remove all traces of detergent

c) Continue rinsing with distilled water being certain to remove all traces of tap water.

d) Sterilize the well with 70% isopropanol.

TEER24 Microplate

Common Electrode Array

How TEER values are determined:

In TEER24 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 (Rtotal = R1 + R2 …) 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.

TEER24 Software Toolbar Icons

Zoom In/Out: zoom in/out on areas of graph

Pan: grab and drag on graph

Data Cursor: Display well information on graph

Select All Wells: Select all wells in well map

Select Well Rows: Select rows in well map

Select Well Columns: Select columns in well map

Select Individual Well: Select wells individually in well map

Clear All Wells: Clear all selected wells in well map

Plot Lines: Display lines across data points in graph

Plot Points: Display individual data points in graph

Display Marks/Pause: Display marks and pause points in graph

Grid Lines: Display gridlines in graph

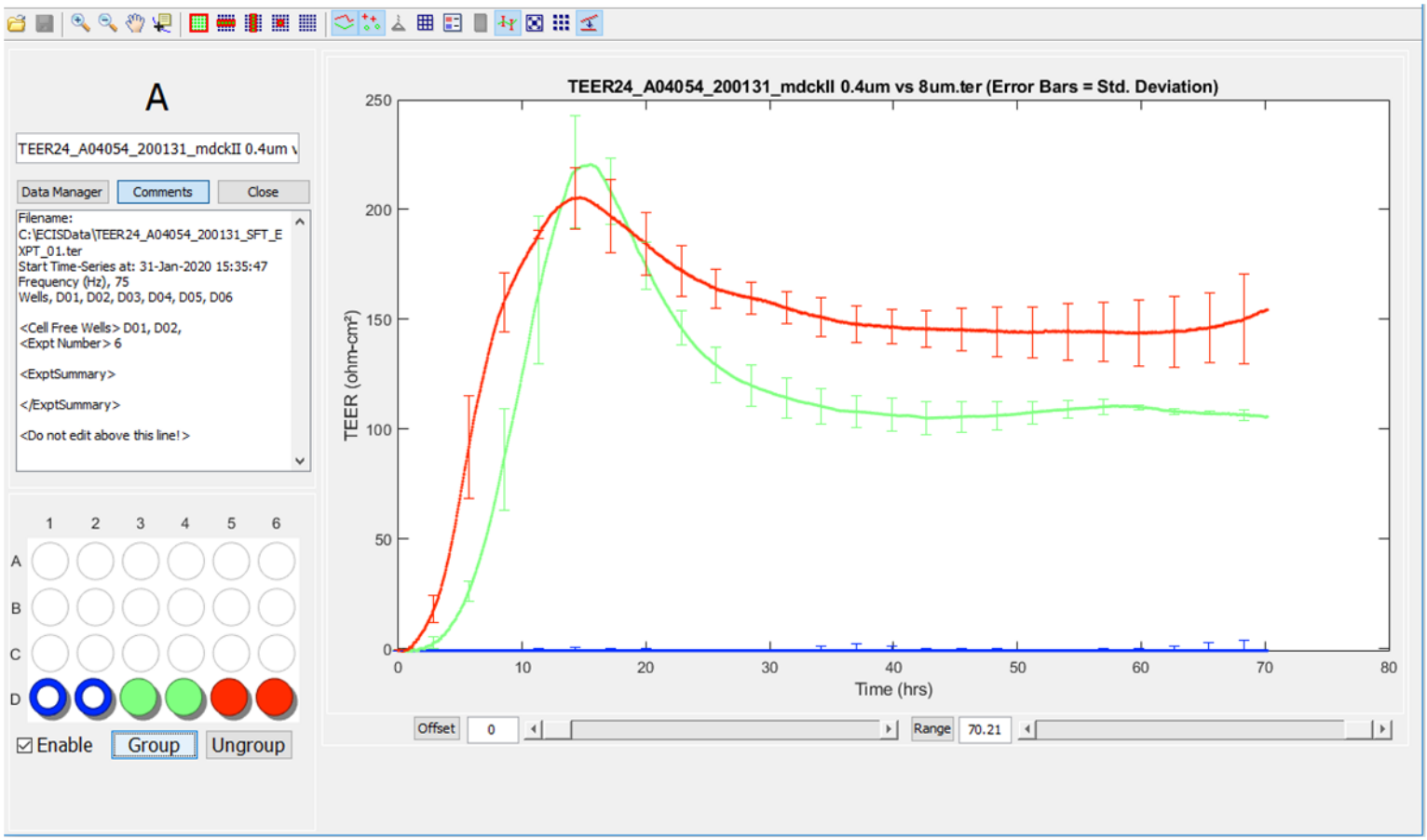
Insert Legend: Insert data legend in graph

Display Error Bars: Display error bars on grouped data

Full Screen: Make graph full screen

Thumbnail View: Display each well data in separate thumbnail

TEER24 Drift Correct: Corrects CellX 0 value to cell-free wells for drift correction



Commands

Commands during data collection:
To pause an experiment during data collection press the **Pause** button.
To resume a paused experiment press the **Resume** button.
To mark a time point as significant press the **Mark** button.

Menu bar commands

Helpful Tips:

1. It is very important to minimize temperature changes during the initial setup and medium exchanges. Insure all solutions are pre-warmed to 37°C.
2. Keep the Common Electrode Array (CEA) in the incubator so that it remains at a constant temperature. The CEA can stand using the guide pins on the back plate of the TEER24 Station keeping the dipping pins suspended and sterile.
3. Before setting the zero-point reference make sure the medium in the TEER24 well assembly is at incubator temperatures. Cold medium will cause the zero-point reference to be set too high leading to negative TEER values.
4. When changing medium in a tissue culture hood use a warming plate to keep the TEER24 well assembly at 37°C.

FILE

Open	Loads a previous experiment.
Recent Files	Loads a recent experiment.
Export TEER	Exports the current experiment to a csv file.
Close	Close current experiment.
Exit	End the program.

EDIT

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

ACQUIRE

Setup New Experiment	Begin new experiment
Activate All Wells	Activates all wells regardless of connection
Find Instrument	Identifies appropriate port for instrument
TEER Parameters	Filter surface area, capacitance, resistance reference.
Validate Assay	Selects the Validation mode of the instrument.
Cell-Free Ref	Allows selection of how Cell-free ref is determined.
Plot Data Rate	Adjusts rate of data collection.

HELP

Manual	Opens PDF version of manual.
Open Log File	Opens the serial log file for inspection.
About	Gives software version and author

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