Applied BioPhysics

TEER24 MANUAL

Contents:

The ECIS® TEER24 uses AC frequencies to determine the TEER value of cell layers on filter substrates.
The measurement consists of two phases; a zero set phase and a data collection phase.
During the zero set phase, the resistance of the ECIS electrodes, solution, and filter is measured and stored in memory as the zero-point reference. During data collection the resistance is measured, and TEER is calculated by subtracting the zero-point reference and scaling for the surface area of the filter.

www.biophysics.com

Applied BioPhysics

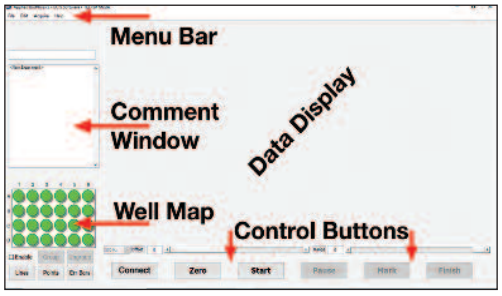
Setting up the TEER24:

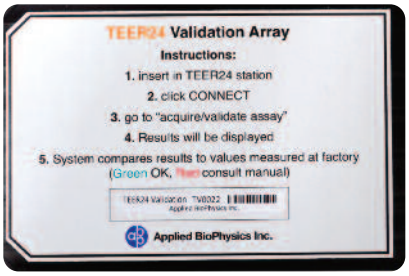
System includes:

- 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
- 2 USB cables

TEER24 Station Controller back

TEER24 Station back





TEER24 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 USB cable from **Unit A** port on back of Station Controller to USB port on back of TEER24 Station.

e. Connect power to laptop and turn laptop on to log in.

f. Enter login with username: **ECIS User** (password not necessary).

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.

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-A** 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.

To Run a TEER24 Experiment (suggested protocol)

1) Incubate TEER24 Station and Common Electrode Array (CEA)

a. Place TEER24 Station in supplied poly bag.

b. Store both TEER24 Station (in poly bag) and CEA in incubator for ~ 1-2 hours for temperature equilibration.

2) Prepare Well Assembly

a. Remove TEER24 Station and CEA from incubator.

b. Follow **System Setup** from "Setting Up TEER24" protocol if system is not setup.

c. Fill TEER24 base plate wells with 1 ml of media.

d. Insert blank transwell filter into each well and add additional 0.2 ml of media to the inner well.

e. Place plate lid on top of baseplate without CEA and clamp into TEER24 Station with proper orientation.

f. Remove lid of baseplate and carefully place CEA with dipping pins into Base Plate using the 3 guide pins to insure proper alignment.

DO NOT PUSH DOWN ON DIPPING PIN ASSEMBLY!

g. Put plate lid back on top of CEA.

*We recommend clamping well plate w/lid in first before inserting CEA to avoid damaging bottom of well plate.

h. Place TEER24 Station w/well assembly back into incubator.

3) Start ECIS TEER24 Software

a. Double-click **TEER24-A** icon to open software

b. Press **Connect** to test connection of each well (shown in the Well Map).

- All wells with medium should appear green; wells without medium or are not connected will appear red.
- If necessary, reseat well assembly and retest connection until desired wells are green.

c. Once TEER24 well assembly has reached equilibrium within incubator conditions, press **Zero** to set the zero reference. A popup window will allow two options:

- **Previous Zero** : A zero reference point from a previous dataset will be loaded.
- **New Zero** : A new zero reference point is recorded and stored.

d. A diagram of the wells and measured TEER values of the cell-free electrodes will be displayed.

e. Once zero reference is set, **Start** button will become available to begin running experiments.

4) Running Experiments

a. Remove well assembly from TEER24 Station.

b. Under aseptic conditions, replace the 0.2ml in the inner well with a cell suspension (30-40,000 cells per 0.33 cm^2 filter area recommended) or replace empty inserts with ones containing cells.

* Two cell free inserts should be included as a control for any non-cell related changes, e.g. evaporation.

c. Return well assembly to TEER24 Station inside incubator.

d. Press **Check** to verify connections.

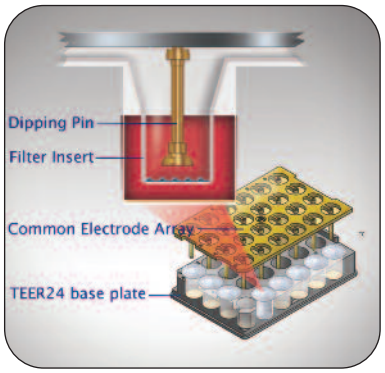
e. Select cell-free wells from the Well Map; a white center will be shown.

f. Press **Start** to begin experiment.

g. During experiment **Pause** may be pressed to pause the experiment for cell treatment, etc.

- After placing well assembly back into the TEER 24 Station, press **Check** to confirm connection.
- Press **Resume** to continue experiment.

h. To end experiment, press **Finish**.



Note: **TEER plate and Filter pre-conditioning.** The ECIS TEER plate electrodes and the filter inserts will experience drift in their electrical properties over time when soaking in medium. The ECIS Electrodes can be stabilized by adding 1.5 ml of 10mM Cysteine solution into each well and inserting the common electrode array in the base plate. Incubate the assembly at RT for an hour or more just prior to use.

Note: The TEER calculation uses a default filter area of 0.33cm^2. To change this value select Acquire > Set Filter Area and enter a new value in the pop-up window.

Note: If you feel that the well plate has lost equilibrium from incubator conditions after removal from and replacement in the station (e.g. cell seeding, treatment etc.), a repeat "Zero" reference function might be necessary after running a "Check" function once the plate has been returned. Also, the initial "Zero" reference function can alternatively be done shortly *after* seeding the cells instead of *before* as recommended in the suggested protocol.

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

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	Close current experiment.
Exit	End the program.

EDIT

Color Palette	Allows editing of well colors.
Group Map	In development.
Error Bars	Selects error bars as SD or SEM.

ACQUIRE

Setup new expt.	Resets the software to run a new experiment.
Activate all wells	Overrides well check to activate all wells.
Find Instrument	Set ECIS COM port and look for Instrument.
Filter Surface Area	Sets the area of the filter. Defaults to 0.33 cm^2.
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.

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.