TEER24 Cell Permeability Assay Whitepaper

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Applied BioPhysics and TEER measurements

Applied BioPhysics has an unrivaled history developing electrical measurements of cell morphology in tissue culture. Beginning with the first description of the ECIS method over 30 years ago by the company founders (Giaever and Keese, **PNAS 81** [1984]), Applied BioPhysics continues to innovate automated cell-based assays. An important aspect of cell morphology that is central to the ECIS measurement is the permeability or inversely the trans epithelial or endothelial electrical resistance (TEER) of cell layers as they are grown upon gold-film electrode substrates. These measurements forgo filters using standard size culture wells greatly simplifying the cell culture process and permeability measurement. A wealth of peer-reviewed literature exists where ECIS is used to report relative changes in cell permeability of both epithelial and endothelial cultures (see www.biophysics.com).

In vitro cell models are only approximations of the *in vivo* environment, and for many permeability studies, growing cells on solid substrates is entirely adequate for the questions being asked. However to get proper cell phenotypes of many epithelial and endothelial cells *in vitro* it is necessary to grow these cells on filters or other porous substrates. Culturing the cells upon porous supports allows the cell monolayer nourishment from both the apical and basal side. In this better approximation of the *in vivo* environment, the cell layer can better polarize and achieve a more *in vivo* like phenotype.


Turn-key system includes: TEER24 instrumentation, PC and software

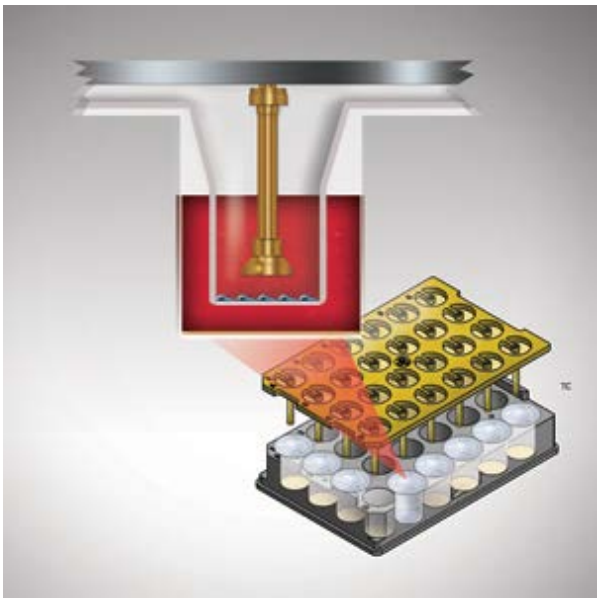
The New ECIS TEER24 Instrument

To facilitate permeability assays that require these more complex cell models we now offer the ECIS TEER24 - a dedicated, affordable filter-based instrument. This new instrument allows researchers to follow the permeability changes in up to 24 cell-covered filter inserts, following real-time changes

taking place in the TEER of cell layers.

Thirty years of monitoring cells as they grow in culture has taught us that cells are extremely sensitive to even slight changes in their environment. Traditional TEER measurements require that the filters be removed from the incubator, causing temperature and pH changes. Some techniques involve transfering the filters to a measurement chamber, thus inducing mechanical stress on the cells as fluid levels change on either side of the filter membrane.

To avoid unwanted environmental changes, the TEER24 with filters remains in the cell culture incubator for continuous monitoring. To further reduce artifacts the Applied BioPhysics measurement only uses gold electrodes, eliminating the presence of silver-silver chloride electrodes and the release of toxic silver ions. Furthermore, the exact placement of the dipping electrode in the TEER24 system affords reproducible results not attainable with imprecisely placed chopstick-style electrodes.



Calculation of TEER

The calculation of the actual TEER value of the cell layer involves separating the contribution of the cells from that of the electrodes, medium, and filter. There are two common approaches to accomplish this - theoretical and empirical. In the theoretical method, the cells, electrodes, medium, and filter are all represented by an equivalent circuit composed of resistors and capacitors (including the constant phase element). The total impedance of this equivalent circuit is measured over a range of AC frequencies and the TEER value calculated.

The empirical method simply uses a low frequency AC signal to first measure the impedance of the electrodes, medium, and filter without cells, and then this value is later subtracted from the measurement including cells. In our experience both methods give nearly equivalent results for cell layers that have TEER values over 10 ohms-cm^2. However, for cell layers with weak barrier function (<10 ohm-cm^2, e.g. many microvascular endothelial cells) we find that the cells are more complex than what is predicted by a simple equivalent circuit, and we favor the empirical method. For these

low values of TEER, the measurement is dominated by the electrode, medium, and filter properties, and the TEER value is a small contributor to the overall impedance. In this instance we optimize the TEER measurement by using higher AC frequency, minimizing the contributions of the electrodes, medium and filter.

Using the ECIS TEER24 Instrument

Using the TEER24 instrument is very straightforward, and the device accepts standard 24 well filter inserts (1 to 24) from any commercial supplier.

1. The first step is to zero the instrument without cells on the filters. Medium is loaded into the sterile wells to be used; the membrane insert of choice is placed in the wells and medium added over the filter. Next the dipping electrode array is added, and the completed assembly is then inserted into the TEER24 device in the culture incubator.

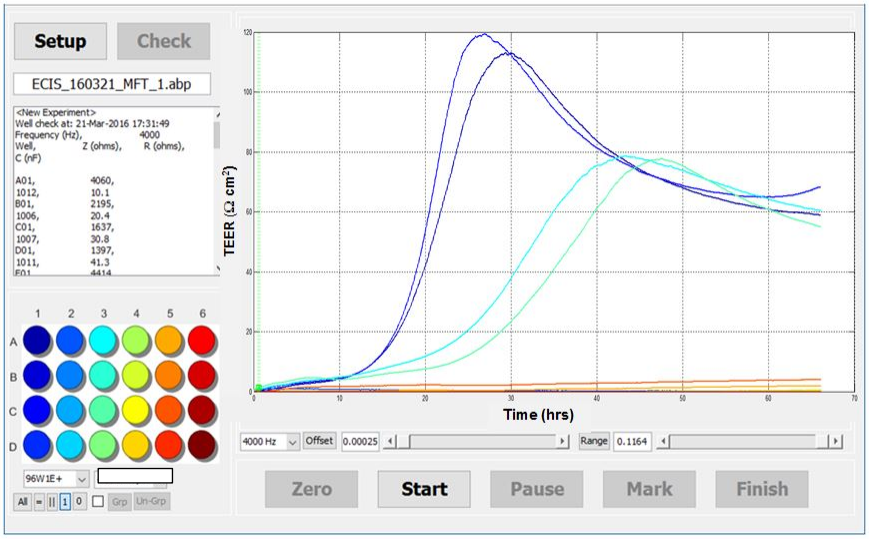
2. After allowing the system to equilibrate with incubator conditions, a quick check is run to assure all connections are good, and then a zero command from the PC touch screen records the filter-only impedance.

3. The system is now ready for the addition of the cell suspension(s). The 24 well plate assembly is removed from the TEER24 Station, placed in the hood, and the filters inoculated with cells.

4. The 24 well assembly is then reinserted into the instrument and the Start button activated to begin the recording of continuous measurements. With all 24 wells being measured, time between points is less than two minutes and even shorter if fewer wells are used.

The graphic user interface will show the changes in each well versus time, plotting the TEER values in ohm-cm^2 units. During data acquisitions one can group and average different wells and call up error bars. In addition, at any time, the data can be copied to a flash drive and analyzed by the TEER24 software that can be installed on another PC.

It's that simple.



The TEER24 graphic user interface collecting data (six wells shown, two without cells). The simple commands can be activated using the touch screen PC provided with the system.