ECIS® trans-Filter Adapter Application Note

For ECIS® Z Theta instruments

using software version 1_2_150 or higher

DO NOT AUTOCLAVE ADAPTER

Introduction

The ECIS® trans-Filter Adapter (tFA) is designed to measure the trans epithelial/endothelial resistance (TEER) of cell layers grown on filter inserts. The tFA accepts up to eight cell culture inserts (24 well size) and is comprised of three parts: (1) the base with contact pads and individually addressable electrodes, (2) the gold-plated stainless steel common dipping electrode array (CDEA) and (3) a clear lid. The individual dipping electrodes screw into the CDEA frame. Before use one should check that all pins are completely tightened to insure each extends the same length into the well.

Cell culture inserts drop into the wells of the base, and the CDEA is placed such that each pin is inserted into the cell culture insert well. Electrical contact to the CDEA is accomplished with three gold pins that project from the base. Contact is assured by magnets in the base that pull down upon the CDEA. The clear lid then covers the wells including the CDEA to keep the contents sterile.

The tFA fits into either side of the standard ECIS® 16 Well Array Stations and two tFAs may be run simultaneously. Alternatively, a tFA and a normal 8W array may also be run together. When asked to identify the array type, choose 8W TEER.

Experimental Protocol

Setup

Preparation of the trans-Filter Adapter

The tFA can be cleaned with any tissue culture compatible detergent and rinsed with distilled water. The device can be sterilized just prior to use by flooding the wells and immersing the tips of the CDEA into 70% isopropyl alcohol or a solution of sodium hypochlorite. We have diluted commercial Clorox® (5.25% sodium hypochlorite) as high as 20% full strength without problems. After treatment the wells and dipping electrodes should be thoroughly rinsed with sterile water and allowed to dry.

Note: the device is not compatible with autoclaving.

Inserting the filter inserts

Cell culture inserts can remain untreated or be treated prior to measurement with matrix proteins; please follow the filter manufacturer's guidelines for these treatments. For TEER measurements we generally use filters having a pore size of 0.4 micrometers diameter.

In this note we are using the Corning Transwell® Permeable Supports (# 3413). Add 1000 microliters of medium in the large outer wells that will be used for the experiment, and with sterile forceps place the membrane inserts into the wells. Add 200 microliters of medium to the inner wells being careful not to damage the filter with the pipet tip.

The media volumes suggested above can be varied somewhat and should be verified when using other commercial brands. The important considerations when filling the wells are that:

- The dipping electrode's hemisphere tip is submerged
- The two compartments are only connected through the filter and never by an overflow between the inner and outer chambers

Assembling the device and Equilibration of the filters

With the filters in place and medium in both inner and outer wells, place the sterile dipping electrodes into the culture inserts with the CDEA frame resting directly on the three support pins. Cover the device with the clear lid and then insert the complete tFA into one side of the ECIS® 16 Well Array Station.

Start the ECIS software and click <setup>. Once the software has identified the ECIS® instrument and the array station, it will check the electrode connections. All wells of the tFA in which you have placed medium should appear green in the lower left hand Well Configuration display. If any wells appear red, check that the tFA is inserted correctly into the ECIS® Array Station and that the medium levels in the wells are high enough to submerge the dipping electrode tip. If necessary add medium to the well.

Select the array type for sides A and B.; for TEER measurements with the tFA select 8W TEER.

Do an electrode check using the default 4000 Hz frequency to become familiar with the open filter readings –for the Corning inserts, values in the range of 100 ohms are common.

At this point we recommend equilibrating the electrodes and filter membranes for 30 minutes or longer in the tissue culture incubator while making ECIS measurements.

Select MFT as the experiment type and the start the data acquisition. Select resistance (R) at 500 Hz as the display mode. Initially resistance may change over time, especially if the medium was colder than 37^{o}C to begin with. Other changes can be due to protein absorption on the electrodes from the medium and fluid leveling between the inner and outer wells.

Once the readings have stabilized, pause the instrument and remove the tFA from the Array Station for cell addition.

Inoculation of cells

To inoculate the filters, remove the 200 microliters of medium in the inner well and replace with 200 microliters of cell suspension added to give the desired cells/cm^2 density.

Sample calculation:

To achieve a final density of 200,000 cells/cm^2

Cell inoculum 0.200 ml
Filter area: 0.33 cm^2

Cells required:
200,000 cells/cm^2 x 0.33 cm2 = 66,000 cells

Cell suspension required:
66,000 cells/0.200 ml = 330,000 cells/ml.

In the experimental setup be certain to include a control membrane insert that does not receive cells, as this is required for final TEER analysis. This cell-free well should be handled in an identical manner, but with the addition of cell-free medium.

Allow 10 minutes for the cells to settle upon the filter and place the tFA back into the 16 wells holder within the incubator space. The filters are now ready for time course measurements.

Data acquisition:

A multiple frequency time course (MFT) can be run with the trans-Filter array. During the final analysis to calculate TEER, the default data presentation will be at the lowest AC frequency which is 62.5 Hz for the standard MFT

frequency set*. At this low frequency the barrier function of the cell monolayer will be revealed, as most current flows through the paracellular path.

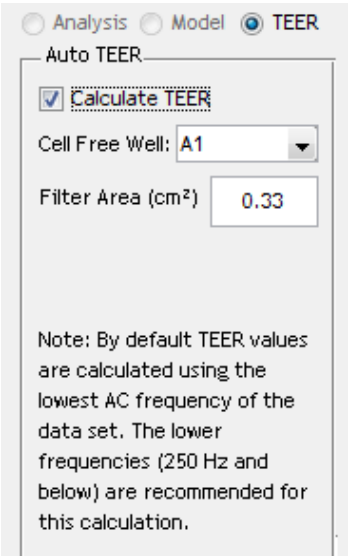
Should you remove the tFA during the experiment remember to pause the measurement and check connections before resuming data acquisition. Unless directed otherwise, the software will do this check at the default 4000 Hz AC frequency.

* *If one elects to use different frequencies for the MFT (available under the Acquire tab) a low frequency from 20 Hz to 250Hz should be included in the list of frequencies. To minimize external noise avoid the AC line frequency of either 60 Hz or 50 Hz.*

Analysis of completed TEER data

Upon completion of the experiment, open the file for analysis. If you did not set the array type to 8W TEER you can do that at this point.

With this array selection, the Auto TEER feature shown below becomes available.



Select the filter without cells (A1 in this case), input the filter area in cm^2 (0.33 cm^2 for the Corning Transwell®) and click on the Calculate TEER box. The system will automatically carry out the calculations and present the TEER values in ohm-cm^2 as a function of time using the lowest AC frequency of the data set.

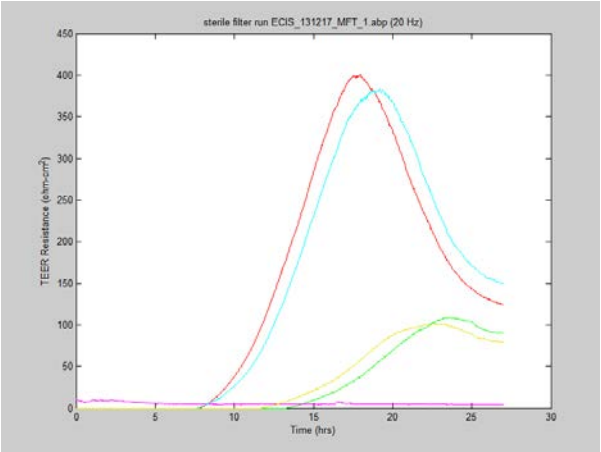
If desired, other frequencies can be examined using the frequency selection feature beneath the left side of the graph.

The graphed data can be stored as a comma separated variable (.csv) file by clicking on the **File** tab, then **Export data** and **Graph Data**.

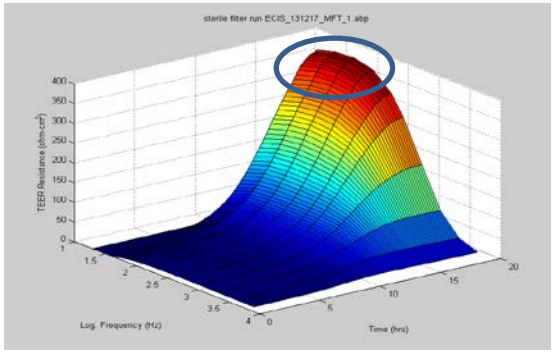
Sample Data

Below is data using Corning® Transwells (0.4 micrometer pores, 10^8 pores/cm^2) with MDCK II cells in complete DMEM with 10% FBS.

The plot is of calculated changes in TEER as a function of time analyzed using the ECIS Auto TEER software.



Wells A1 (black) and A5 (magenta) are both cell-free. At time zero the other filters received an inoculation of MDCK II cells at two different concentrations. The red and blue traces and the yellow and green traces are duplicate runs at 1.0X and 0.5X cell concentrations respectively.



A 3D plot of the first 20 hours of the run is shown looking at the data from the blue trace (1.0X inoculation). Notice how the four lowest frequencies (circled) are in good agreement with curves peaking around 370 ohm-cm^2. As frequencies get higher, the TEER that is reported appears lower, as more current now capacitively couples through the cell membranes rather than flowing though the paracellular path.

Please contact us should you have any questions.