

# ECIS® 8W and 24W Trans-Filter Adapter Application Note

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For ECIS® Z Theta instruments  
software version 1\_2\_210 or higher

***Note: the complete trans filter adapters can now be autoclaved;  
bleach sterilization is not recommended***



## 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 the tFA 24W accepts up to twenty four cell culture inserts. Both units are 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.

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

The 8W tFA fits into either side of the standard ECIS® 16 Well Array Station 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 Filter** for the tFA. The 24WtFA fits into the 96 Well Array Station. When asked to identify the array type, choose **24W Filter**.

## Experimental Protocol

### Setup

#### Preparation of the trans-Filter Adapter

Both the 8W and 24W tFA can be cleaned with any tissue culture compatible detergent and rinsed with distilled water. It is recommended to sterilize the device by autoclaving.

Alternatively, one can flood the wells and expose the CDEA to a 70% isopropyl alcohol solution. After this treatment the wells and dipping electrodes should be thoroughly rinsed with sterile water and allowed to dry.

*Note: do not sterilize using bleach (sodium hypochlorite) solutions.*

#### 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).

Pipet 1000 microliters of medium in the large outer wells that will be used for the experiment and, using sterile forceps, place the membrane inserts into the wells. Pipet 200 microliters of medium into the inner wells being careful not to damage the filter with the pipet tip.

The media volumes suggested above can be varied somewhat and may be different when using other commercial brands of membrane inserts.

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. Place the lid on the device and then insert the complete tFA into the Array Station.

Open 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 Array Station and that medium levels in the inner wells are sufficiently high to submerge the dipping electrode tip. If necessary add medium to the inner well.

Select the appropriate array type and 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 start the data acquisition. The system will automatically select 250 Hz as the default mode for display of R. Initially resistance may change over time, especially if the medium was colder than 37°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 finish data collection and remove the tFA from the Array Station for cell addition.

## Inoculation of cells

To inoculate the filters, remove the 200 microliters of medium from the inner well and replace this with 200 microliters of warm cell suspension to provide the desired cells/cm<sup>2</sup> density.

Sample calculation:

To achieve a final density of 200,000 cells/cm<sup>2</sup>

Cell inoculum 0.200 ml  
Filter area: 0.33 cm<sup>2</sup>

Cells required:  
 $200,000 \text{ cells/cm}^2 \times 0.33 \text{ cm}^2 = 66,000 \text{ cells}$

Cell suspension required:  
 $66,000 \text{ cells} / 0.200 \text{ ml} = 330,000 \text{ cells/ml}$ .

**In the experimental setup be certain to include a cell-free membrane insert that receives medium without cells as this is required for TEER analysis.**

Allow 10 minutes for the cells to settle upon the filter at room temperature and then place the tFA back into the Array Station within the incubator space. Run **Setup** and verify as before that all wells being used are properly connected. The filters are now ready for time course measurements.

## Data acquisition:

Data should be acquired using the multiple frequency time course (MFT) program. As in the equilibration of the filters, the system will automatically select 250 Hz as the default mode for display.

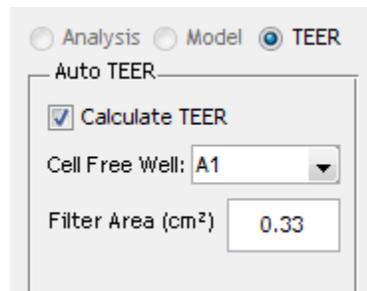
Should you remove the tFA during the course of the experiment, remember to first pause the measurement. After placing the tFA back in the Array Station, be certain to check connections before resuming data acquisition. Unless directed otherwise, the software will do this check at the 4000 Hz.

*\* If one elects to use other than the default frequencies for the MFT (available under the Acquire tab), a low frequency from 125 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 data file for analysis. If you did not set the array type to 8W Filter 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  $\text{cm}^2$  ( $0.33 \text{ cm}^2$  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  $\text{ohm-cm}^2$  as a function of time using the 250Hz frequency of the data set.

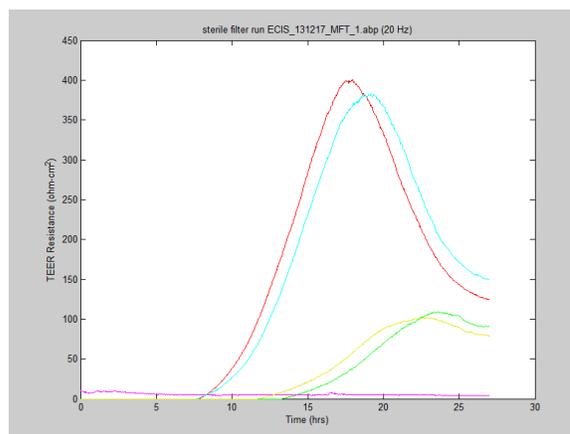
If desired, other frequencies can be examined using the frequency selection feature beneath the left side of the graph.

*Note: the reported TEER values will fall off at high frequencies as more current will flow capacitively through the cell membranes rather than through the paracellular space.*

Graphs can be exported (under **Display Options**) or the graphed TEER data can be stored as a comma separated variable (.csv) file by clicking on the **File** tab, then **Export data** and **Graph Data**.

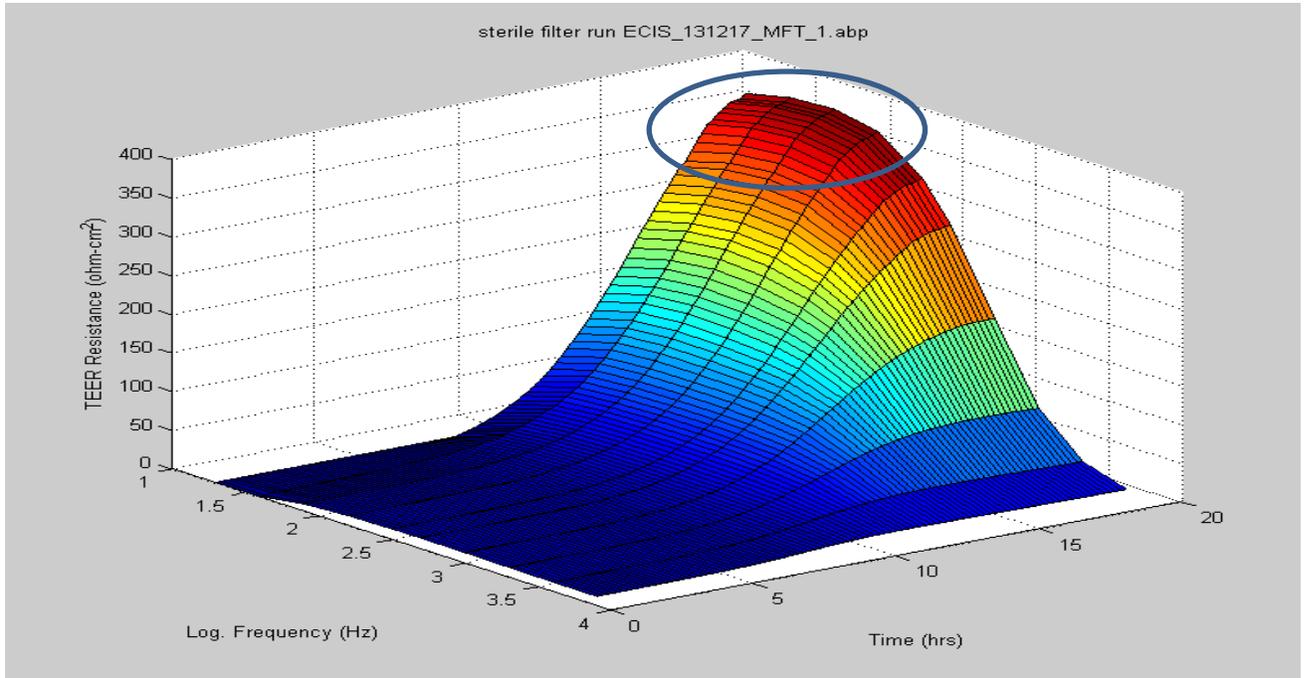
## Sample Data

Below is a plot of data from an 8 Well tFA adapter using Corning® Transwells (0.4 micrometer pores,  $10^8$  pores/ $\text{cm}^2$  cat # 3413) with a heavy inoculation of MDCK II cells in complete DMEM with 10% FBS.



The plot shows 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<sup>2</sup>. As frequencies get higher, the TEER that is reported appears lower, as more current now capacitively couples through the cell membranes rather than flowing through the paracellular path.*

**Please contact us should you have any questions.**