Stabilization of Electrodes to Maximize Repeatability

Applied BioPhysics

Quantifying Cell Behavior

The stabilization of electrodes is very important for experiment to experiment repeatability. Before inoculating wells and running ECIS experiments, electrodes should be stabilized to minimize electrode drift during experimental runs and establish good reproducibility from one experiment to another.

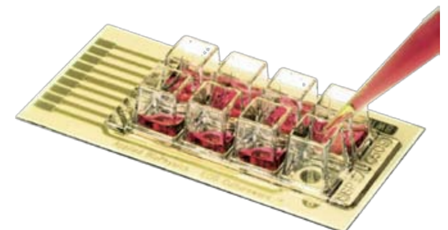
Newer ECIS instruments have a feature that conditions the electrodes electrically before they are inoculated. This feature can be used by itself or in conjunction with cysteine treatment, but cysteine treatment is generally not needed with instrument-stabilized electrodes.

Follow the steps below to stabilize electrodes prior to inoculation. Some of the steps are optional and are labeled as such. Also, if specific protein coats are going to be applied, they must always be adsorbed before the electrode is exposed to complete, serum containing, culture medium.

1. Cysteine treat the wells (optional see ECIS manual for details)
2. Pre-coat electrodes with protein to be used (optional see manual for suggestions).
3. Add 200 microliters of complete medium to each well and place the array in the array station. Click the **Setup/Check** button to ascertain the array is properly connected.
4. Run electrode stabilization.
5. An electrode **Check*** (optional) can now be run to check the impedance value of the cell-free wells**.**

Following electrical stabilization, it is recommended to let the electrode equilibrate in medium at least 15 minutes before inoculation to allow impedance values to settle.

*[1] Upon checking the impedance values of the empty electrode you will notice the electrode stabilization procedure has elevated the capacitance and reduced the resistance of the electrodes to that associated with freshly etched or cysteine-treated arrays. Following electrode stabilization, the 1E well capacitance will normally be over 5 nF and over 50 nF for the 10E and 10E+ arrays. If you are planning to study the attachment and spreading of cells and these values have not been achieved, you may elect to rerun the Stabilize electrode feature before inoculation.



Inoculation of Arrays

Careful inoculation is the key to good reproducibility from well to well. This is especially important if you are measuring the cells on a single electrode that has a 250 micrometer diameter electrode to monitor cells (e.g. 1E arrays). Careful inoculation is also important in cell proliferation experiments where 10E or 10E+ arrays are being inoculated with less than confluent cell populations (usually 20% to 50% of confluent numbers).

It is most important to obtain a uniform inoculation of the well bottom. The wells should have an even “snowfall” of cells so that each electrode has approximately the same number of cells falling on its surface. This can be facilitated by following three important guidelines:

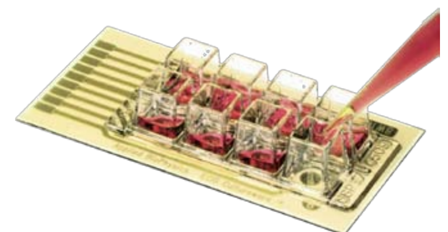
1. The cell suspension should be monodisperse.

For some cell lines this is easily achieved, but for others, particularly if cells have been attached and spread for long periods of time, clumping takes place and longer trypsinization may be needed. The goal is to achieve is a clearly monodisperse cell suspension. It is also important to keep the cell suspension well mixed and uniform so each well receives approximately the same number of cells.

2. Pre-warm cell suspensions.

Temperature considerations can also contribute to uneven cell distribution. If the temperature of the cell suspension is lower than the temperature of the incubator, when placed in the incubator, the wells will be heated from the bottom. This will cause a convection cell to form, where medium rises in the center and falls back down the walls of wells. Because of this flow, as cells attempt to land in the central region of the well, they are swept upward. The overall effect is that the cell density becomes reduced in the central regions of the well, and a confluent layer forms at the well perimeter but not over the centrally located electrodes. This is very undesirable, especially with the 1E arrays.

This problem can be avoided by making sure the array and the cell suspension are at incubator temperature. We have had success using cell suspensions warmed a degree or two higher than incubator temperatures (for example 38 C for a 37 C incubator) to eliminate the convection cell entirely.



3. Avoid mixing the cell suspension with liquid already in the well.

If possible, it is best to remove all medium from wells before adding the cell suspension so no mixing within the wells is required (see suggested cell numbers below).

If a cell suspension must be added to medium already in the well, mix the two solutions thoroughly being careful to not get liquid on the upper perimeter of the well. If liquid gets on the lip of the well it may form a seal with the lid and prevent the wells from freely exchanging gas in the incubator space.

Should medium finds its way to the top of the well, carefully and aseptically remove the liquid or arrange an alternative cover for the array (e.g. a sterile petri dish cover) until the liquid evaporates and the normal lid can be used.

Cell numbers to achieve confluent monolayers (please note: these numbers can vary considerably depending on the actual cell type being used).

The base of an 8 well array has a surface area of approximately 0.8 cm^2. A typical inoculation to give a confluent cell layer is approximately 10^5 cells per well. To obtain an even coating of cells at 10^5 cells per well in 8 well arrays, we suggest preparing a monodisperse cell suspension at approximately 2.5 x 10^5 cells /ml and adding 400 microliters per well.

The 96 well array has a surface area of approximately 0.3 cm^2 and needs an inoculation of 0.4 x 10^5 cells per well for a confluent layer. To obtain an even coating of cells at 10^5 cells per well in 96 well arrays, we suggest preparing a monodisperse cell suspension at approximately 1.3 x10^5 cells per ml and adding 300 microliters per well.

These volumes and the inoculation density are only guidelines and can be changed without problem. However, well volumes below 150 microliters could interfere with current flow, so it is best to have at least 200 microliters in the wells during measurements.