Charlie's Guide to Cell Inoculation

Careful inoculation is the key to good reproducibility from well to well and from experiment to experiment. It is most important to obtain a uniform inoculation of the well bottom. To accomplish this, wells should receive 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 attentively following some important guidelines:

1. Make up a monodisperse cell suspension.

   Θ Note: 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 suspension of single cells.

2. Keep the suspension agitated

   Θ Note: It is also important to keep the cell suspension uniform so each well receives approximately the same number of cells; agitate the cell suspension frequently to prevent settling of the cells.

3. Add the suspension to an empty well

   Θ Note: If possible, avoid mixing the cell suspension with liquid already in the well. It is best to remove all media from wells before adding the cell suspension such that no mixing within the wells is required. If a cell suspension must be added to liquid already in the well, thorough mixing of the two solutions is essential.

4. Allow the cells to settle and attach with the array outside of the incubator

   Θ Note: Temperature considerations are often overlooked and can be extremely important when dealing with cell distribution in small wells.

   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. Due to this flow, as cells attempt to fall to 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. This is very undesirable and critical with the 1E arrays and 10E arrays with their centrally located measuring electrodes.

   Here are two ways to deal with this problem

   (a) Simply inoculate the ECIS® arrays outside of the incubator using room temperature medium, and then wait 20 to 30 minutes before placing the array in the incubator space. Since there is no heating from below, there is no thermal convection, as the cells settle uniformly over the entire substrate and begin to attach to the surface. We recently started using this approach, and the results have been most satisfactory. CO_2 dependent medium would experience some pH increase out of the incubator in this protocol, and one could consider ways to avoid this. In our hands, however, we have not found a pH drift to be a problem, as once in the incubator space, we observe normal cell attachment and spreading impedance data.

   (b) Alternatively, if solution #1 cannot be used, we then recommend using a cell suspension and array that are pre-warmed to incubator temperature and immediately place the array into the incubator upon inoculation. The goal again is to prevent thermal convection by having no heating of the wells taking place during cell settling.

Cell numbers

If one wishes to achieve confluence after attachment and spreading we recommend the following inoculation guidelines. The numbers below are approximate and dependent on the actual cell line or primary cells used in the array and the viability of cells in the suspensions used for inoculation.

For 8W standard arrays the substrate area is 0.81 cm^2. When completely confluent with cells, this surface will have ≈100,000 cells. By adding 400 microliters of a cell suspension at 2.5 x 10^5 cells/ml to each well, one should achieve confluence upon attachment and spreading of the cells.

For 96W standard arrays the substrate area is 0.3 cm^2. When completely confluent with cells, this surface will have 40,000 cells. By adding 300 microliters of a cell suspension at 1.2 x 10^5 cells/ml to each well, one should achieve confluence upon attachment and spreading of the cells.

For cell proliferation measurements, or if one wishes cells achieve a natural confluence upon contact inhibition, lower concentrations of cells can, of course, be used for the cell suspension. For example, using 4 fold less cells and assuming a 24 hours generation time, cell confluence should be achieved in ≈ 2 days post inoculation. For 10 fold less cells, confluence would be achieved in ≈ 3 to 4 days post inoculation.