

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 250 micrometer diameter electrode (1E arrays) or performing cell proliferation experiments, where wells are being inoculated with sparse cell populations.

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 following three important guidelines:

1. Make up a monodisperse cell suspension.

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 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 aid in obtaining an even 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. This is very undesirable, especially with the 1E arrays with their centrally located measuring electrode.

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 media from wells before adding the cell suspension so 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.