ECM Coating of ECIS® Arrays

ECIS® arrays shipped from Applied BioPhysics have no macromolecular coatings. When culture medium is added to the arrays, proteins and other large molecules in the medium immediately adsorb to the very wettable gold surface as they do with any uncoated tissue culture dish. The wide use of animal serum in cell culture protocols creates a situation where the protein composition coating the cultureware substrates can be highly variable and undefined.

Rather than allowing serum proteins to coat the substrate, including the ECIS® electrodes, Applied BioPhysics highly recommends that researchers coat ALL cultureware with an ECM protein appropriate for the cell used. This will aid in enhancing reproducibility in ECIS® assays and compatibility between ECIS® assays and other cell-based techniques.

Θ Note: Remember the first macromolecules the substrate is exposed to, will be the final coating that the cells first encounter. With this in mind below are the suggested sequence of steps to prepare the array for inoculation with a defined protein coat.

Best Practice for ECIS® Array Prep

1. Coat all wells with predefined ECM protein
2. Rinse the wells with sterile ddH_2O three times
3. Fill the wells with 200µl of sterile 10mM cysteine solution
4. Incubate the array at room temperature for a minimum of 30 minutes
5. Rinse the wells with sterile ddH_2O three times
6. Add 200 µl of complete medium
7. Run electrical stabilization
8. Remove the medium and add the cell suspension

Coating Protocols

For the convenience of the user we provide protocols below for fibronectin, gelatin, collagen, and laminin.

Fibronectin; Adapted from Sigma (www.sigmaaldrich.com) for human derived fibroblast secreted fibronectin.

1. Slowly thaw the fibronectin solution at 2 - 8°C. Do not vortex or shake vigorously to resuspend the fibronectin as this will cause the fibronectin to "crash" out of solution, which is irreversible.
2. Dilute the product in sterile Hank's Balanced Salt Solution (HBSS). Dilutions vary with each application but routinely fall in the range of 1–10 µg/ml.
3. Fill each well with 200 µl of the diluted fibronectin solution.
4. Incubate at 37°C for 1–2 hours.
5. Wash 3 times with sterile HBSS and plate the cells.

Gelatin; Adapted from ATCC (www.atcc.org) for 0.1% gelatin solution

1. Add 100 µL of 0.1% gelatin solution to each well.
2. Place the array in a 37°C incubator (with or without 5% CO2) for at least 30 minutes and up to overnight.
3. Aspirate the excess gelatin solution from the ECIS® array.
4. Add 200 µL of complete growth medium to each well.
5. Place the gelatin coated ECIS® array in a 37°C, 5% CO_2 incubator for at least one hour to equilibrate before inoculating with the cell suspension.

Collagen; Adapted from ibidi (www.ibidi.com) for rat tail type I collagen.

1. Dilute collagen to 25 µg/ml using17.5 mM acetic acid. Θ Note: Collagen is insoluble at neutral pH.
2. Fill the wells with 100 µl of the diluted collagen.
3. Incubate at room temperature for one hour.
4. Fully aspirate the wells.
5. Carefully rinse with ddH_2O or serum-free medium.

Laminin; Adapted from Santa Cruz Biotechnology (www.scbt.com) to coat at 5µg/cm^2.

1. Dilute laminin to 50 µg/ml using sterile, serum-free culture medium.
2. Add 100 µl to each well.
3. Incubate at room temperature for one hour.
4. Aspirate unbound material and rinse gently using serum-free medium. Plates are now ready to use.