Compound Addition

The ECIS® measurement is extremely sensitive, and so even small responses of the cells to temperature, shear stress, etc. are usually detectable. When adding compounds minimizing these unrelated responses is necessary to maximize the biological effects of the compounds. However, there are two opposing physical factors influencing compound addition to the ECIS® wells; Disturbing the cells due to the shear stress of fluid flowing across the cells and achieving rapid mixing by properly agitating the fluid in the well.

Optimizing a compound addition will depend on the trade-off between rapid mixing and disturbing the cells. In general, compounds that take a long period of time to be effective can better tolerate disturbing the cells as the physical effects from compound addition is generally short lived. These compounds should be added in a tissue culture hood in large volumes of pre-mixed of 2x solutions. In contrast when adding compounds whose effects are rapid, the physical effects of the addition must be minimized within the constraint of diffusion. These compounds are added in the incubator with just enough volume to get reproducible results ≈ 20 - 50 µl.

Θ Note: The viscosity of the compound being added will greatly determine the final volumes and rigorousness of mixing required for reproducible experiments. The key issue at work is that unless the compound is completely mixed, the cells will experience an initial effective concentration of the compound different from the calculated concentration.

Adding Compounds in a Tissue Culture Hood

The following protocol will minimize the physical response of the cells while delivering a rapidly mixed compound:

1. Prepare the cells for addition of the compound of interest by changing the medium over the cells using the medium (or balanced salt solution) that will be present when the compound is added.
   (a) Press Pause on the ECIS® software.
   (b) Remove the ECIS® arrays and place them on a 37°C heated surface in a tissue culture hood.
   (c) Aspirate the medium from the wells.
   (d) Add 400µl of experimental medium to each well.
   (e) Return the ECIS® arrays to ECIS® holder.
   (f) Press Resume in the ECIS® software.
2. Wait a minimum of 4 hours to allow the cells time to adjust and equilibrate to the experimental conditions.

   Θ Note: Cells should recover from the physical act of being removed from the incubator, manipulated and put back in the incubator in about 2 - 4 hours. Responding to changes in medium composition, e.g. serum deprivation, may take much longer.
3. Prepare the compound or compounds to be tested at 2X the final desired concentration in the same solution as is in the wells. For each well into which a compound is to be added prepare 200µl of solution. A control solution of medium plus vehicle should be prepared in the same exact manner.
4. In containers with loose fitting lids put the compound solution(s) in the incubator for a minimum of one hour to insure temperature and gas equilibration.
5. Press Pause on the ECIS® software.
6. Remove the ECIS® arrays and place them on a 37°C heated surface in a tissue culture hood.
7. Aspirate 200µl of the experimental medium from the wells.
8. Add 200µl of the compound solutions to each well.
9. Plunge the pipettor 1 - 2 times slowly to completely mix the compound
10. Return the ECIS® arrays to the incubator and ECIS® holder.
11. Press Resume in the ECIS® software.

Adding Compounds in the Incubator

The following protocol allows for the careful addition of the compound solution in the incubator. By only adding volume to the wells it reduces the amount of time the incubator door remains open.

1. Follow steps 1 - 4 of the preceding protocol substituting for step 3 20µl per well of a 21x concentration of the compound(s) of interest. If a little more volume is needed to get adequate mixing of the solution, 40µl at 11x or 50µl at 9x concentration can be used.
2. Once solutions have reached thermal and gas equilibrium with the incubator environment open the incubator door, remove the lid to ECIS® array A and add 20 (40, 50) µl of solution to each well.
3. Replace the lid to array A and repeat for array B.
4. Close the incubator door and continue collecting data until the experiment is finished.

Θ Note: The researcher should optimize the volume of solution added by testing volumes and concentrations until maximum reproducibility between wells and experiments are achieved.