Tips for Establishing Successful Cell-Based Assays: Part 3

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Cell Culture Maintenance and Propagation

For successful and reproducible cell culture assays, it is imperative that cells be maintained and propagated in a consistent manner to minimize genetic and non-genetic changes due to selection, genetic drift or contamination. Typically, cell medium and propagation schedule information accompanies the cell purchase. In general the conditions described below should be used initially. Be sure to keep a cell log on each cell type and collect information such as medium and dissociation solution, passage number and any other observations. Typically, the general maintenance protocols are as follows for adherent cells.

1. Warm media and dissociation solution. The dissociation solution could be trypsin or a non- enzymatic dissociation solution such as Cell-Stripper, Accutase, Hyqtase, Detachin to name a few. A 37°C water bath can be used to warm the solutions prior to use.
2. Take the flask containing cells out of incubator and observe color and clarity of the medium. If the cultures are contaminated, the medium may appear opaque or yellow if phenol red is used in the medium. Be sure that there are no white shiny mold colonies on the bottom. If contamination is observed and confirmed microscopically, tighten the lid and dispose the flask by autoclaving. Another option is to open the flask outside of the culture area and treated thoroughly with bleach and pour down the sink once the medium turns white. Cap the flask and dispose as normal. Be sure the disposal of contamination is carried out away from active cell culture room so as to not contaminate other cell lines.
3. Always examine cells under microscope to determine health, confluency and contamination. This is a critical step. Check for any changes in cell morphology and look for clumping. Do not split cells without having gone through this step.
4. In the hood, remove medium from the flask either by aspiration or by pouring it off into a chlorox containing beaker. Only work with one cell line at a time. Do not aspirate from multiple cell lines with the same pipette.
5. Wash cells once with 5-10 ml of a calcium and magnesium free Phosphate Buffered Saline (PBS). Note that calcium and magnesium are absent as they are key mediators of cell attachment.
6. Remove PBS and add enough dissociation solution to cover the cell monolayer.
7. Incubate 2-5 minutes or until cells begin sloughing off the flask at either room temperature for lightly adherent cells such as HEK293 or in the incubator for more adherent cells such as HepG2. Monitor the cells under microscopes.
8. If several minutes have gone by and the cells are still stubbornly stuck to the plate, you can either add more dissociation solution or incubate longer.
9. Tap or hit the flask from the side to dislodge the cells from the bottom.
10. Add at least 10 x volume of medium to cells and pipette carefully without creating bubbles to break up cell clumps as described above and transfer an aliquot to the culture

vessel. Non-enzymatic solutions will not generate uniform single cell suspensions without this step.

11. Measure volume, count cells and transfer an appropriate aliquot to a new flask containing fresh culture medium.
12. Monitor the cells under microscopes after each step.

Suspension Cultures

It is trickier to see if suspension cultures are contaminated. Look for changes in color of culture medium or changes in the appearance of the cells themselves, such as more debris that is indicative of contamination and treat contaminated culture as described above.

There are a couple of ways to split suspension cultures. a. Count the cells in an aliquot of the cultures. Determine cells per milliliter and transfer an aliquot containing the appropriate number of cells to a new flask with fresh medium so that the final cell density desired is achieved. b. Another way is to simply transfer cells/medium to fresh medium at 1:5, 1:10 or 1:20 split ratio depending on the cell growth rate. This is risky in that the exact cell density is not controlled. c. A final way is to centrifuge the entire culture or a portion of that culture, remove the old media, fully resuspend the cells in a small amount of new culture medium, count cells to determine cell density and dilute a fraction of the cells into a larger volume of culture media in desired final cell density in a new flask.