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Tips for Establishing Successful Cell-Based Assays: Part 1

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Cell-based assays are a critical part of the workflow for discovering new chemical entities. They are used throughout the drug discovery process from target validation, primary and secondary compound screening to determining the safety and selectivity of new leads. Since they are ubiquitously used, it is with utmost importance that one chooses appropriate cells, assay conditions and designs, and data analysis. The following sections on cell-based applications provide general guidance in designing robust cell-based assays for screening and lead optimization.

Requirements for Identifying and Developing the Right Cells

1. Cells have appropriate secondary signal transduction pathways
2. Early passage cell lines are available and free of mycoplasma contamination; strategy for mycoplasma screening is established.
3. Using stably transfected cells
    1. Plasmid sequence has been verified; message or protein expression is confirmed.
    2. Level of expression is appropriate for the activity required for testing by functional screening of clones with various expression profiles.
4. Using transiently transfected cells: appropriate cell line and transfection procedure have been demonstrated with reproducibility.
    - Plasmids available and have passed sequence and restriction mapping QC criteria.
5. Native cells
    - Expression of target has been verified and signal transduction pathways are present.
6. Phenotypic assay
    - Cells must be physiologically relevant.
7. QC guidelines must be established and sample preparation must pass QC each time the cells are plated.
    - QC could be for viability or appropriate levels of control response in the assay among other specific parameters determined for your project
8. Preliminary sources for all chemical and biochemical reagents have been identified and if running a screen, there are enough materials in the same lots available to complete the screen and secondary testing. Be sure to reserve some of the initial lot to test the new lots against, if needed.
9. Cell culture methods and assay protocol should be available as written SOP (standard operating procedure). This includes the number of cells, dilution factor (e.g. 1:10), passage numbers, passage frequency, activity stability as a function of cell passage, and optimum cell density for target activity.
10. Biological activity is target specific as demonstrated by pharmacology of tool compound activity in target-transfected cells vs. vector-control-transfected cells or parental cells.

11. Assay signal is dependent upon number of cells present.

12. Preliminary data should show a saturated activator and/or inhibitor response with sufficient signal window.

Source of Cells: Cells can be obtained from vendors or laboratories as either primary cells or cell lines as frozen ampoules or as pre-plated cells. Be aware that some of the cell lines are patented, and although you can purchase them, the seller may not be granting the right to perform studies with them. The diligence work is up to the buyer.

There are several procedures to follow once the cells reach your facility. If received as pre-plated cells in culture, they should be placed in an isolation incubator (for all new arrivals regardless of source) for growth until it is confirmed that the cells are free of contamination such as mold, bacteria and mycoplasma. The culture medium and split ratio should be those recommended by the vendor unless new conditions have been validated. Once the cell line has been determined to be free from contamination, it can be moved to a non-isolation incubator. It should be noted that the best practice to move cultures from one incubator to another, is upon splitting the cells in a fresh flask to avoid incubator-to-incubator transfer resulted contamination. Once cells have been verified to be free from contamination again, they should be amplified and frozen into several vials to create an initial master cell-bank. Cells received from frozen stock should also be placed into the isolation incubator upon thawing until deemed contamination free.

Where can you obtain primary cells?
Primary cells can be obtained from several vendors or one can create the cells in-house as needed. The products sold include the cells as well as optimized media and may contain some validation information on the cells. Cells have usually been tested for contaminants. Some media offered do not have lists of ingredients, as they are proprietary. Be aware that the ingredients in media are not inert and may alter the activity you wish to measure. There are several options for vendors of primary cells and the list change frequently. Lonza and ATCC are good places to start.

Where can you obtain cells lines?
There are multiple vendors for cell lines such as Multispan Inc, a premier GPCR assay cell line provider: www.multispaninc.com.

Of course, it is now possible to purchase cells that are for immediate assay use in “Assay Ready” format as reagents. In this type of assay, the cells are thawed and placed immediately into the multi-well assay plates for evaluation. These cells cannot be passaged as they are division arrested by chemical or radiation methods and may be sold as part of a kit using the vendor’s proprietary technology. However, if these cells need an incubator, it should be an isolation incubator to prevent contamination of the other cell lines in the lab.