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# Managing Cell-Based Potency Assays – from Development to Lifecycle Maintenance

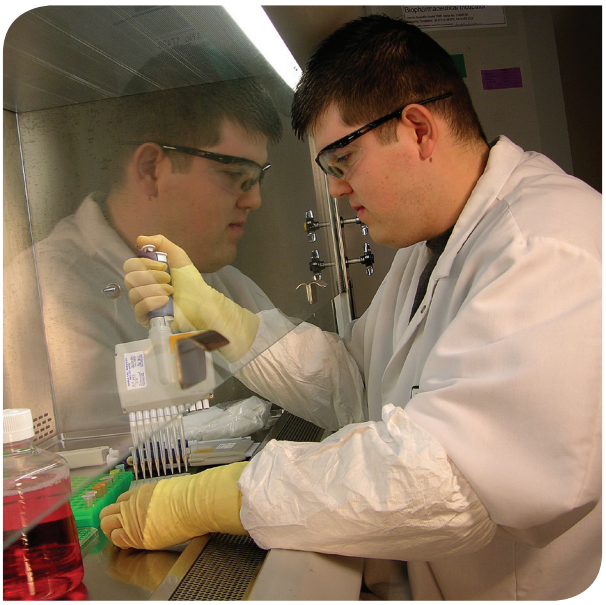
by Dr. Weihong Wang, Technology Development Manager

## Introduction

Characterization of a biological product, which includes the determination of physicochemical properties, biological activity, immunochemical properties, purity and impurities, is necessary to establish the safety and efficacy profile of a given product (ICH Q6B). Cell culture-based potency assays are often the preferred format for determining biological activity since they measure the physiological response elicited by the product and can generate results within a relatively short period of time unlike typical animal-based assays. Cell-based potency assays are also often the only functional assays used for product release and stability programs. Other uses of cell-based assays include qualification of internal reference standards; characterization of process intermediates, formulations and degradation products; and support of changes in the product production process.

## Types of Cell-Based Potency Assays

Various types of cell-based assays, based on the mechanism of action of the product, have been used to support product licensing and commercial release. Some common types of cell-based assays used to characterize recombinant protein/monoclonal antibodies include proliferation assays, cytotoxicity assays, reporter gene assays, cell surface receptor binding assays and assays to measure induction/inhibition of functionally essential protein or other signal molecule (such as phosphorylated proteins, enzymes, cytokines and cAMP). Most commonly, results from cell-based potency assays are expressed as "relative potency" as determined by comparison of test samples to the response obtained for the reference standard.



## Developing a Cell-Based Potency Assay

When developing a cell-based potency assay, careful consideration needs to be taken in selecting the appropriate cell line. First and foremost, selection of the cell line should be based on the mechanism of action of the product. Optimally, the cell line should be from a lineage close to the cell/tissue type targeted by the drug. For example, osteosarcoma cell lines are often selected for cell-based assays that test drugs expected to affect bone morphogenesis. A surrogate cell line may be used if an appropriate cell surface receptor is expressed (either endogenously or via stable transfection) and the assay readout reflects the well-defined mechanism of action. For example, CRE-Luciferase reporter gene assays are commonly used for testing GPCR modulating drugs. Another important factor in selecting cell line is its growth characteristics. A cell line intended for use in a QC assay should be a homogeneous population that has stable and robust growth over a reasonable period of time and/or passage numbers. The cell passage procedure should be well defined and followed in order to ensure consistent assay performance. Last, but not least, the selected cell line should be able to respond to the drug consistently in a dose dependent manner when appropriate assay conditions are developed. The dose titration curve should be optimized so that the dilutions are appropriately distributed throughout the entire dose response curve with sufficient coverage in the linear portion of the curve. Once the cell line is selected and well characterized, a cell bank should be created and tested to ensure the validity of the assay and consistent performance throughout the lifecycle of the method. Cell bank characterization should include, at minimum, microbial testing

(bacteria, fungus, mycoplasma), growth characteristics using well-established cell culture conditions and functional stability.

Other factors that need to be considered when developing a cell-based potency assay include, but are not limited to, the choice of reference standard/critical reagents, definition of system suitability parameters and implementation of appropriate statistical analysis. A reference standard could be an established national (e.g., USP) or international (e.g., WHO) standard or a well characterized in-house material. Selection of critical reagents, such as fetal bovine serum, is also critical for successful assay development. When developing cell-based assays, important assay parameters, such as signal to noise ratio, relative standard deviation (RSD) between replicates, etc. needs to be monitored and if necessary, optimized using design of experiment (DOE) studies. Finally, appropriate statistical analysis needs to be applied when generating final relative potency results. Currently, parallel line analysis (PLA) is considered to be a standard methodology for calculating relative potency. PLA calculates relative potency results by comparing the response generated by the test sample vs. reference standard over the entire dose titration curve rather than relying on response comparison of one single dose pair. PLA also generates statistical value(s) that measure parallelism between the reference standard and the sample dose response curve. Several statistical theories, such as F-statistics, Chi-square statistics (both Difference Tests), as well as various Equivalence Tests, have been applied for measuring parallelism in bioassays. There has been much debate in the bioassay field on the pros and cons of each of these methodologies. The data analysis software deploying PLA function can either be developed in-house (when statistical expertise is available) or off the shelf. Several commercial software programs that support PLA are available for cell-based assays and immunoassays. The commonly used ones include Softmax Pro (Molecular Devices), PLA (Stegmann Systems) and StatLIA (Brendan Technologies).

## Transferring a Cell-Based Potency Assay

After a cell-based potency assay is established, additional considerations need to be made with regard to method transfer to a different laboratory, such as a contract testing organization. To begin, allow sufficient time for method transfer. In the case where a qualified cell bank has not been generated, creation and testing of the cell bank itself can take weeks to months. In addition, methods established for R&D purposes may not be suitable for QC testing, therefore time needed for method optimization should be taken into consideration. Also keep in mind that establishing a cell-based potency assay in a different lab often takes more than just providing the SOP. Each cell line has its own unique characteristics. Proper cell culture handling technique greatly influences the performance of the assay. If problems occur, on-site visits and training are usually most effective in identifying and resolving the issue.

## Assay Maintenance Post Validation

Once a bioassay has been validated and implemented for routine testing, it is important to monitor its performance over time. This can be accomplished by maintaining a trending chart for suitable parameters of the reference standard response curve and potency of analyzed QC samples. Maintaining and periodically performing statistical analysis of this chart help to identify the presence and source of assay drift at an early stage. Assay performance trending, along with appropriate reference standard and critical reagent qualification, prevents significant assay drift over time and ensures reliable results over the lifecycle of the assay.

## Conclusion

Cell-based potency assays are an important part of establishing safety and efficacy profiles of biopharmaceutical products. Due to the nature of cell-based potency assays, method development, method transfer and long-term assay maintenance can often be quite challenging. Careful considerations, both scientifically as well practically, need to be taken to ensure a successful outcome.