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In-Cell ELISA Assays to Assess Drug Relative Potency Using the Cytation Imaging Reader for Sample Analysis

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PURPOSE

Potency assays are crucial characterization tools for almost all biotherapeutics during clinical and pre-clinical development for quality control and market authorization purposes. These potency assays must be tailored to the drug mechanism of action (MOA) and provide a specific readout related to the drug's biological properties. Many formats exist for measuring drug potency (i.e. ELISA, flow cytometry, western blot, etc.) and should be chosen based on the platform that can successfully show accurate potency of a particular drug candidate. Here we show the utility of the In-Cell ELISA assay platform to assess relative potency of multiple drug therapies using the Cytation10 and Cytation3 cell imaging readers. The Cytation reader is a useful tool for relative potency measurements because it allows for fully automated microscopy (brightfield, fluorescent and confocal) in a multi-well plate reader format that standard plate readers cannot provide. In combination with Gen5 software versions such as Image+ and Image Prime, the user can efficiently analyze data like a standard plate reader with the added imaging benefit that is necessary for an In-Cell ELISA. Both readers can produce image-based potency readouts but vary in their analytical functionality and data analysis settings based on software versions within Gen5. Image and data treatment are important components in the implementation of these methods and the advantages, utility, and considerations of these are discussed here using two case studies. One involves protein expression as a function of cell number while the second uses the reversal of protein aggregate formation as functional end points.

OBJECTIVE

The objective of these projects was to utilize the Cytation Imaging Reader to develop multiple assays to measure relative potency of various drug products for sample analysis testing. We hoped to show the versatility and accuracy of this instrument in measuring different drug dose response outputs to obtain relative potency curves that could be used for drug stability studies during preclinical development.

METHODS

Target cells were treated with varying doses of the drug product (DP) of interest. Cells were either dosed with a DP reference material or DP test sample to determine relative potency. The cells then reacted to the DP relative to the dose level and the reaction was measured through an In-Cell ELISA using the Cytation Imagers. Cellular reactions included protein expression or seeded aggregate reduction in response to increased drug dosing. The response was read on the Cytation imagers to produce dose response curves based on fluorescent intensity relative to cell count. Relative potency was determined based on the curve comparison between the DP reference and test sample. Cytation10 with Image Prime software was used to measure potency of protein expression based on the secondary masking feature. Secondary masking allowed for the quantification of cells expressing the protein of interest and total number of cells to determine changes in protein expression with changing dose concentrations. Cytation3 with Image+ software was used to measure aggregate formation based on the primary masking feature. Primary masking allowed for the quantification of cells that were aggregating and cells that were not aggregating to determine aggregation with changing DP dose concentrations.

RESULTS

- Measurement of protein expression and aggregate formation using the In-Cell ELISA allowed for the determination of relative potency between reference and test sample relative to the drug concentration using 4-PL curve fitting (Figure 1 and Figure 2).
- Evaluation of parallelism between the reference and sample curves was used to determine the best curve fit in both cases. Asymptote ratio, slope ratio and R^2 were evaluated to determine which settings produced accurate and reproducible curves.
- The best curve fit for potency accuracy when measuring protein expression was found on the Cytation10 with Image Prime software capable of secondary masking. A single image was taken at each dose level and a primary mask was applied to the nuclear fluorophore (total cells) and a secondary mask was applied to the fluorophore representing the protein of interest to determine total cellular response to drug dosing (Figure 3).
- To create the best curve fit for potency accuracy when measuring aggregate formation on the Cytation3 using Image+, a nine-image stitch was taken at each dose level and two primary masks were applied to the nuclear fluorophore (non-aggregated cells) and the fluorophore representing the aggregated cells to differentiate the two (Figure 3). Both assays have been characterized and are validation ready.
- Initial linearity analysis of the protein expression In-Cell ELISA suggests the ability to test the drug concentration within a range of 50-200% (Figure 4)

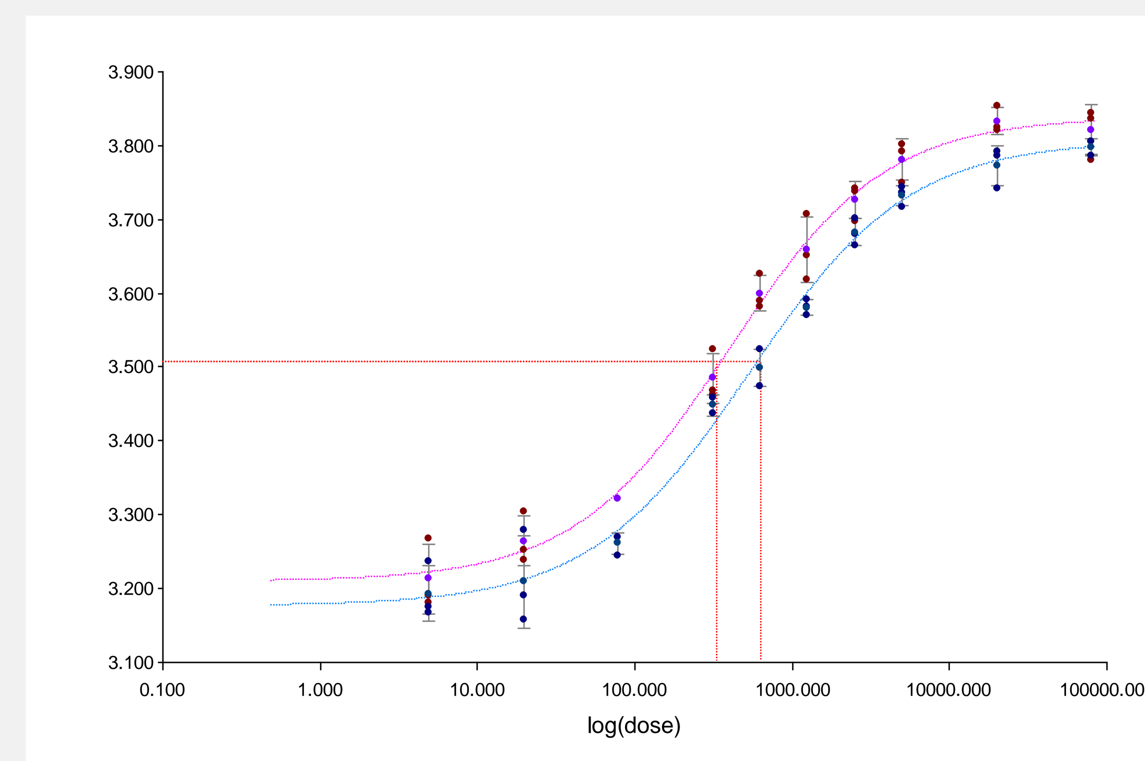

Figure 1: Relative Potency of reference vs. test sample based on protein expression

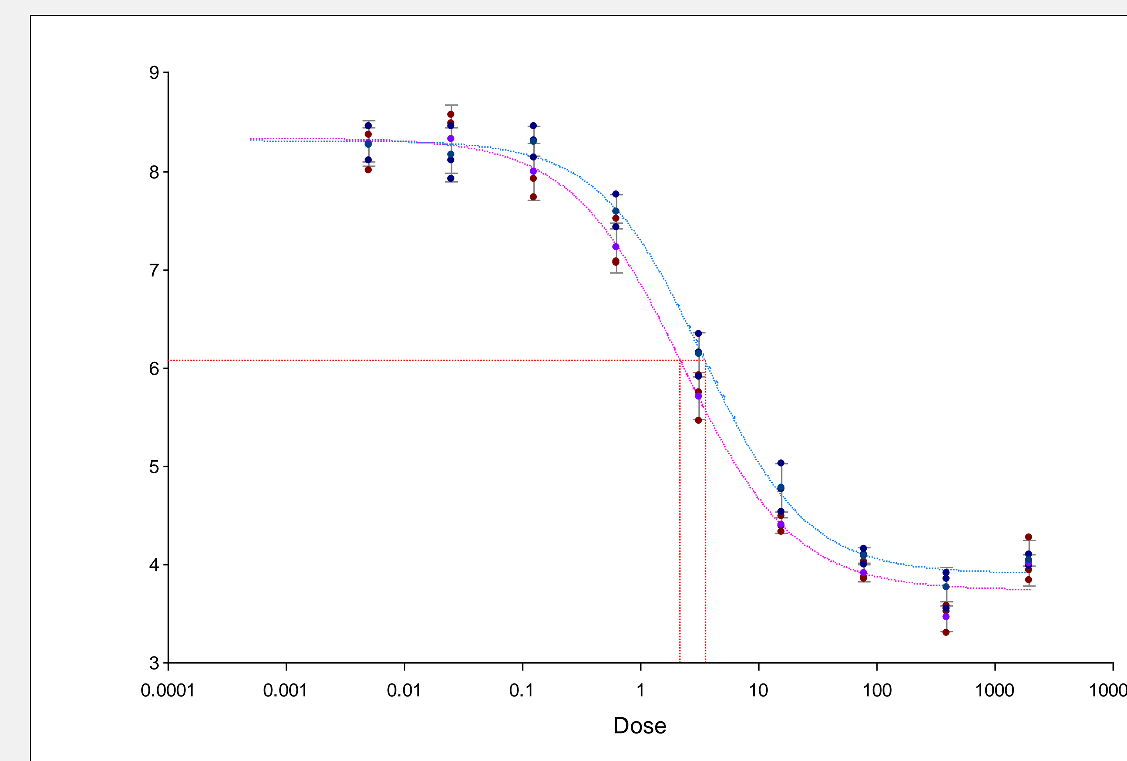

Figure 2: Relative Potency of reference vs. test sample based on aggregate formation (inhibition)

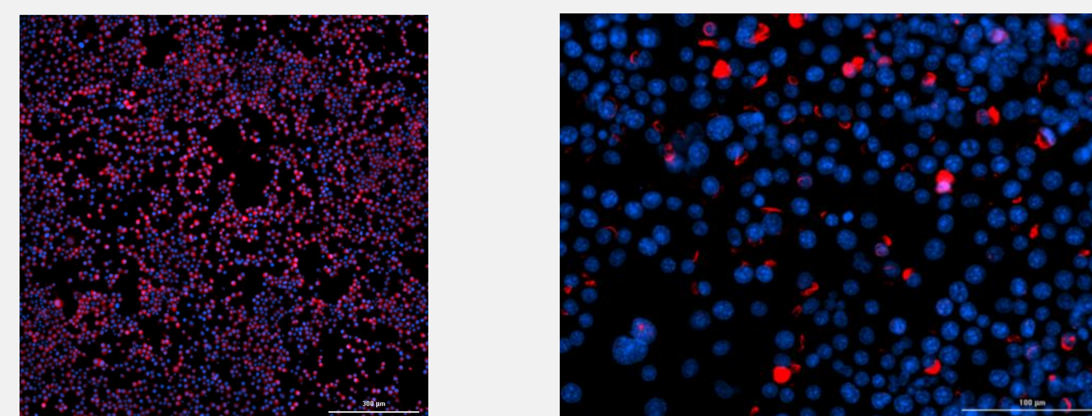

Figure 3: Protein expression (left) using secondary masking and aggregate formation (right) using primary masking

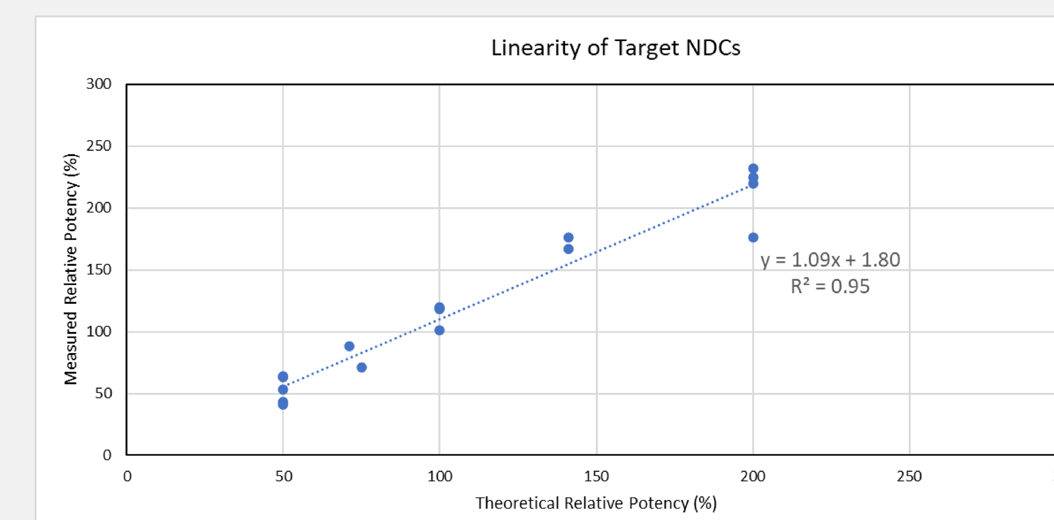

Figure 4: Preliminary linearity analysis from 50-200% NDC of the protein expression based In-Cell ELISA

CONCLUSIONS

- An In-Cell ELISA using the Cytation imager provides the framework for a potency assay capable of assessing DP relative potency if the MOA involves a direct cellular response such as protein expression or cell aggregation.
- This technique can successfully be used to show relative potency for In-Cell ELISAs that show both increasing and decreasing dose response curves in response to drug stimulus.
- The choice of instrumentation and software will depend on the readout and available software settings which provide the most accurate potency curves.
- We have shown the use of Cytation instrumentation to measure drug product relative potency, however, this technique could extend to other materials such as drug substance.
- This assay platform also shows the potential to create qualified potency assays based on its ability to measure relative potency accurately over a broad dose range.
- Overall this technique addresses unique MOA needs that may otherwise be unmet or inadequate in their biological relevance due to its unique imaging-based measurement capabilities.

REFERENCES

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