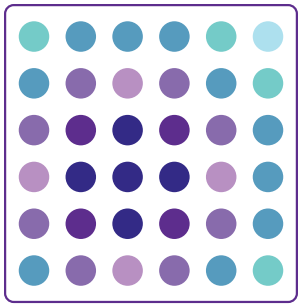
Catalent development



**CASE STUDY**

# Modernization of a Cell-Based Potency Bioassay

## Executive Summary

A client sought Catalent's expertise to improve an existing bioassay for assessing the potency of the client's biopharmaceutical therapeutic molecule. The existing bioassay was not precise, accurate, robust or very rapid. Assessment and quantification of potency for biopharmaceutical moieties is often done using cell-based bioassays. When deriving potency values for a given test sample, in a bioassay, a full dilution dose/response curve of the reference standard is compared to that of the sample. Potency of the sample is relative to the Reference Standard (which is set at 100% potency). Essentially, the degree of transposition of the sample response curve, compared to the Reference Standard curve, is used to calculate relative potency. As opposed to physicochemical assays that provide values of weight/volume (e.g., mg/ml), bioassays provide values of relative activity/weight (e.g., % of reference potency, or units/mg).

For a bioassay to provide an accurate measurement of relative potency, certain built-in checks, or assay acceptance criteria need to be satisfied. The dose/response curves (generally a 4-parameter fit logistic) need to have definable upper and lower asymptotes. The Reference Standard curve and the Test Sample curve have to be sufficiently parallel, with similar upper and lower asymptotes (statistically testable). Only then can the derived sample potency be determined accurately. Further, it is desirable that an independent "Assay Control" sample dose/response curve be tested alongside the Reference Standard and the Test Sample. This would ensure that the assay, itself, was executed correctly.

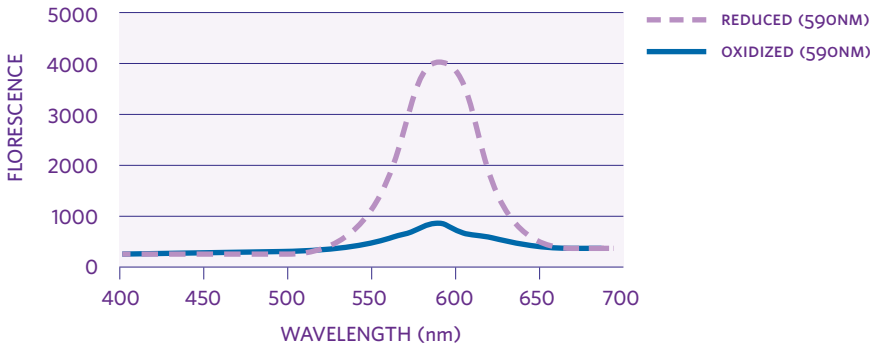
A two-phased strategy was employed to improve the bioassay. First, rather than AlamarBlue™, an alternative system, CellTiter-Glo™ was used to measure cell survival/proliferation. For the second part of the strategy, the assay was optimized for both maximum dose/response curves and even response (no edge-well effect) across all 96 wells of the microtiter plate. The original assay, AlamarBlue, was highly variable, with low throughput (inner 60 wells), and an unacceptable failure rate. The Catalent alternative assay system, CellTiter-Glo, was able to utilize all 96 wells, and was accurate, robust and reproducible with a failure rate of < 5%. Further, it was able to support a full-curve reference and two samples, along with a full-curve individual plate assay control, all in triplicate on one plate. The new assay proved to be rapid, robust, accurate and precise and assisted the Catalent client in meeting their late phase and commercial needs.

DEVELOPMENT DELIVERY SUPPLY

## The Challenge

The existing assay was based upon the ability of the client's therapeutic molecule to support the growth/proliferation of a "responder" cell line. The proliferation of the responder cells was quantified using a vital Red/Ox dye, AlamarBlue. AlamarBlue functioned by being reduced by an active electron transport system in viable, metabolizing cells. Upon reduction, the AlamarBlue was converted from a blue substrate to a fluorescent product, measurable in a microplate-based fluorometer. The assay was characterized by high variability, low throughput and lengthy assay duration (five days). The assay results were subject to variables as high as 20-30%, and intrinsic assay failure rates were as high as 40%. Additionally, due to the lengthy incubation/stimulation time (four days), plus incubation time with the AlamarBlue (12-16 hours), microplate cell culture edge-well effects were seen (common to long incubation times in microtiter plates), forcing use of only the inner 60 wells of a 96-well plate. This resulted in low assay throughput: only one test sample per plate. Further, assay controls were executed as "point values" (four wells of 100% positive induction and four wells of 0% induction), but no full dilution curve of assay control material, as would be far more desirable.

**FIGURE 1** AlamarBlue (oxidized and reduced) fluorescence signal




Catalent was challenged to provide a significantly improved, more precise, accurate, robust, reproducible and rapid bioassay that was essential in meeting the client's upcoming late phase and commercial needs.

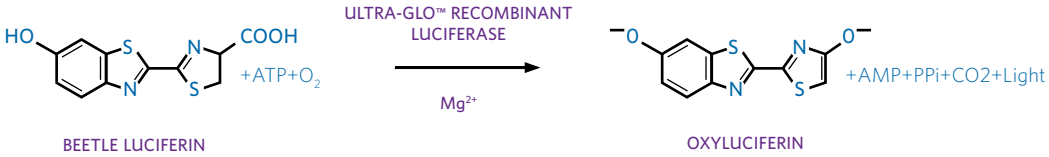
## The Catalent Solution

To address the challenge, Catalent implemented a two-phased strategy. First, rather than AlamarBlue, CellTiter-Glo was used to assess cell proliferation. Unlike AlamarBlue, which required uptake by cells in order to access the electron transport, CellTiter-Glo quantified the total amount of ATP that had been generated by proliferating cells (downstream of electron transport) following lysis of those cells with a gentle detergent. The signal output was chemiluminescent, which could be quantified by using a microplate-based luminometer.

Luminescence has a greater dynamic range and sensitivity than fluorescence. The luminescent signal is proportional to the amount of ATP present, which is directly proportional to the number of viable, actively metabolizing cells that had been present in culture. Thus, a dose dependent curve was

generated upon stimulation with the therapeutic molecule (ligand) and the bioactivity could be quantified and expressed as a relative potency. After as few as 48 hours, the level of produced ATP could theoretically be assessed with a 30 – 60 minute incubation with CellTiter-Glo. The assay was developed with the aim of developing a 48 hour assay. With shorter assay duration, on one plate, a full curve of reference sample, 2 test (unknown) samples and an assay control sample could be tested, in triplicate.

**FIGURE 2** CellTiter-Glo Reaction




In the second part of the strategy, the assay was optimized for both maximum dose/response curves and even response (no edge-well effect) across all 96 wells of the microtiter plate. **FIGURE 3** shows the results of the CellTiter-Glo plate uniformity tests, either 48 or 72 hour stimulation, in a visual format.

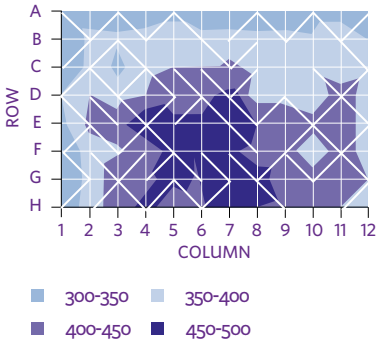
Both the 48-hr stimulation plate and the 72-hr stimulation plate demonstrate readily identifiable plate position effect, or lack of uniformity. As a result, potency of reference, sample or control would be inaccurate. A test of potential parameters impacting plate uniformity was conducted. The final result was that the two critical parameters proved to be sufficient equilibration of assay plates to room temperature prior to CellTiter- Glo addition and maintenance of cells in suspension in the source trough prior to transferring the cells to the microtiter plate. The final result may be seen in the plate uniformity map shown in **FIGURE 4**.

As shown in the figure, optimization of the critical parameters was effective at spreading out the RLU response evenly throughout the plate.

The plate sample map (sample positioning) was further optimized via "Center-weighted Plate Mapping" to prevent any "curve hook" artifacts.

**FIGURE 3** Initial Plate Uniformity Maps, 48 hour and 72 hour stimulation

2 Day Culture, 80% Ligand Stim




3 Day Culture, 80% Ligand Stim

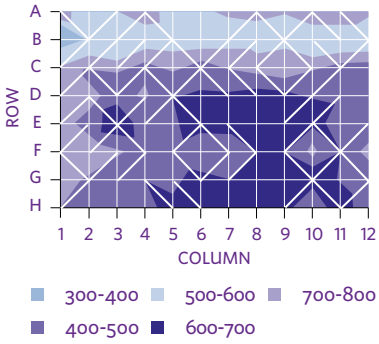



**FIGURE 4** Final Plate Uniformity Map

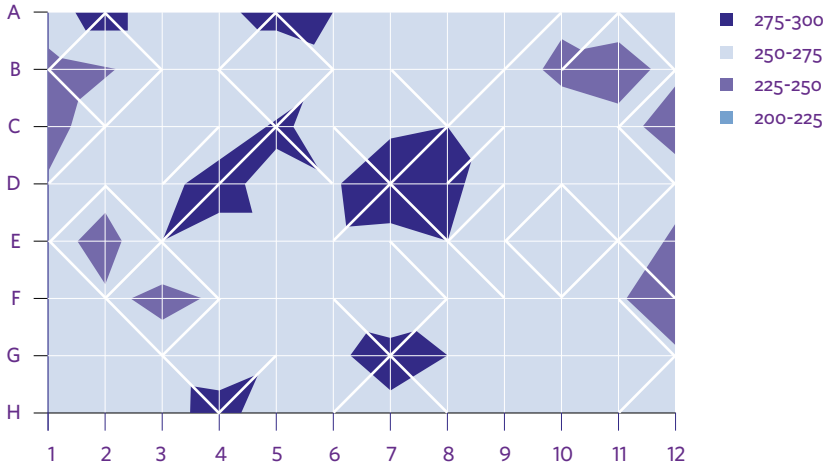


FIGURE 5 shows that the use of a center-weight sample map, in addition to the other optimized assay parameters discussed above, result in CellTiter-Glo dose/response curves that are optimally defined by 4-parameter fit logistics.

FIGURE 5 Center-weight map dose/response curves from 4 independent preparation of reference standard, as performed by two independent analysts.


## Conclusion

The development and optimization of a two-day bioassay, capable of accurately assessing potency of a therapeutic molecule in terms of an end-point cell survival response, has been described above. The original assay, AlamarBlue, was highly variable, with low throughput (inner 60 wells), and an unacceptable failure rate. The Catalent alternative assay, CellTiter-Glo, was able to utilize all 96 wells, was accurate, robust and reproducible, with a failure rate < 5%. Further, it is able to support a full-curve reference and two samples, along with a full-curve individual plate assay control, all in triplicate on one plate.

Whereas the original bioassay would have presented a significant (and perhaps unacceptable) level of risk for use in support of late phase/registration lot/commercial release and stability, the new assay was rapid, robust, accurate and precise, easily meeting the stringency required for such work.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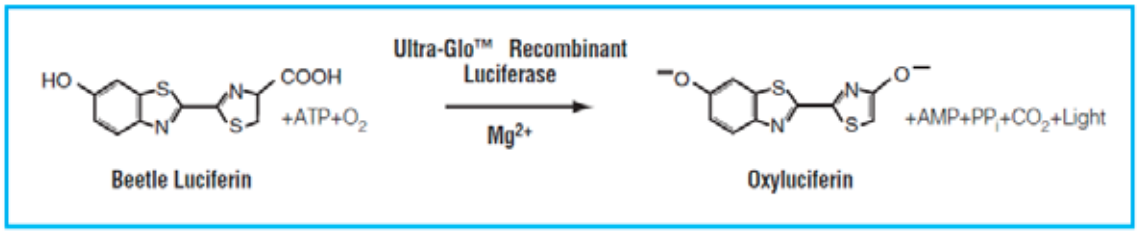


CellTiter-Glo reaction

Figure 2