A Simple Method to Determine Relative Potency (EC_{50}) Using Octet® BLI Technology

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Introduction

Unlike small molecule assays where the active concentration of a drug substance can be considered to be directly in line with the total concentration prepared; biological assays have the inherent issue that the total protein concentration determined using absorbance or a dye-binding assay often does not correlate well to the observed response.

This variation in active protein concentration is especially prevalent when considering cell line development, where although total protein yield is a major factor the binding parameters of individual clones and their active concentration is the main consideration when determining the optimal clones to progress and in a recent study, ~32% of monoclonal antibodies were found to contain one or more additional heavy or light chains, which degrades the desired properties of the antibody.[1] Therefore, in order to determine how much of the biologic is correctly folded and active, a bioassay (potency assay) is performed.

A potency assay is a quantitative measure of biological activity; it must represent the intended biological effect (mechanism of action) and, where possible, be related to the clinical response. Potency assays help provide assurance of the quality and consistency of the product, and common applications include the characterization of antibodies, vaccines, therapeutic proteins, cell and gene therapies in manufacturing, stability testing, and lot release.[2]

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Classically, Bio-Layer Interferometry (BLI) assays have been used to observe the response of the analyte (in solution) binding to the ligand (attached to the biosensor). This assay format may present issues when using kinetics and the global affinity to determine potency as the kinetic association rate constant (ka) is dependent upon the active protein concentration, and as such, the global affinity value (KD), and a potency assay may be more suitable. Due to the variability in a biological assay, a measurement of activity of the result of the test material relative to a known standard is more suitable and is known as relative potency (RP). Relative potency assumes that the reference and test material are biologically similar and that the test material behaves like a concentration or dilution of the standard.[2] Real-time Octet® BLI assays provide several benefits over standard endpoint assays such as ELISA. Direct, label-free assays substantially reduce assay development time compared to endpoint assays as there is no need to choose secondary antibodies, which may cross-react with capture molecules and require labeling optimization due to indirect detection. Total assay and hands-on times can be substantially reduced thanks to the Octet® BLI Discovery and Analysis Software where traditional long washing and incubation steps can be removed, which frees up more time for additional experiments. Key advantages of real-time, label-free Octet® BLI assays are that low-affinity interactions can be elucidated more easily due to the removal of wash steps, and kinetics and affinity of each interaction can be determined for each interaction, unlike endpoint assays from which critical association kinetic information is missing and only affinity can be calculated.
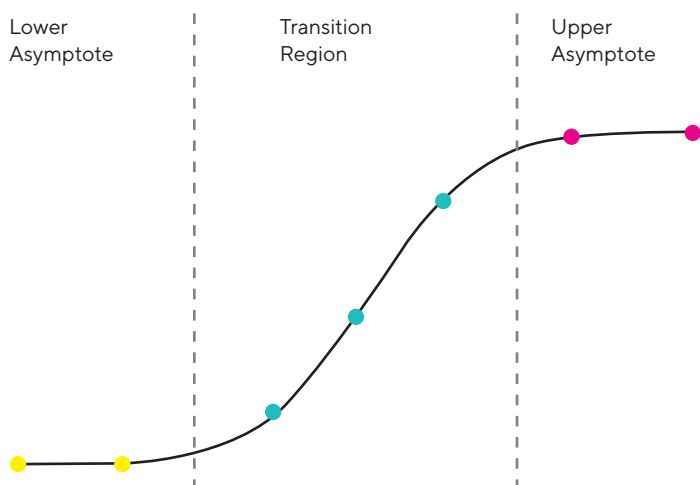
Octet® BLI Discovery and Analysis Software now contains a new module for quickly and easily assessing relative potency using the same assay format currently used for quantitation and kinetic assays, allowing you to measure EC_{50} similarity using a range of parameter logistic equations (PL), linear and semilog lines. This document introduces basic assay design and an introduction to the new software module.

Appropriate Assay Design

When designing a relative potency assay, a sigmoidal response (EC_{50}) is preferred if saturation of the dose response is possible. Sigmoidal responses for relative potency calculation should contain at least seven concentrations of the test material and span the lower asymptote, transition region, and upper asymptote (Figure 1).

Designing and setting up a relative potency assay is simple using the experiment wizard where Dose Response and Custom Dose Response modules have been added in addition to Quantitation, Kinetics, and Epitope Binning modules. Dose Response assays are similar to quantitation or advanced quantitation assays but the analyte concentrations of test materials are known in advance while Custom Dose Response assays are similar to standard kinetic assays.

Several fully customizable two and three-step basic assay templates are included for Dose Response, and these follow a simple naming convention (Figure 2). A two-step assay contains a sample and detection step, whereas a three-step assay contains an additional loading step for the biosensor. For example, the 2_step_8CH_96W_1TestAndStandard_7conc method is a 2-step method for an 8-channel system using a 96-well plate that contains one test material and one standard, both with seven standard analyte concentrations (number of samples in a column plus a buffer blank).

Figure 1 *Using the Potency Module in Octet® BLI Discovery and Analysis Software*

Lower Asymptote | Transition Region | Upper Asymptote

The workflow of the assay is easily observed in the plate definition tab and modified by clicking on the modify button. The concentrations for the standard and the test material can be entered manually or by highlighting the concentration column and right-clicking to 'Set Well Data' (Figure 3).

Prior to performing the assay, the method can be reviewed in the Review Experiment Tab by simply clicking or sliding the slide bar to view each step.
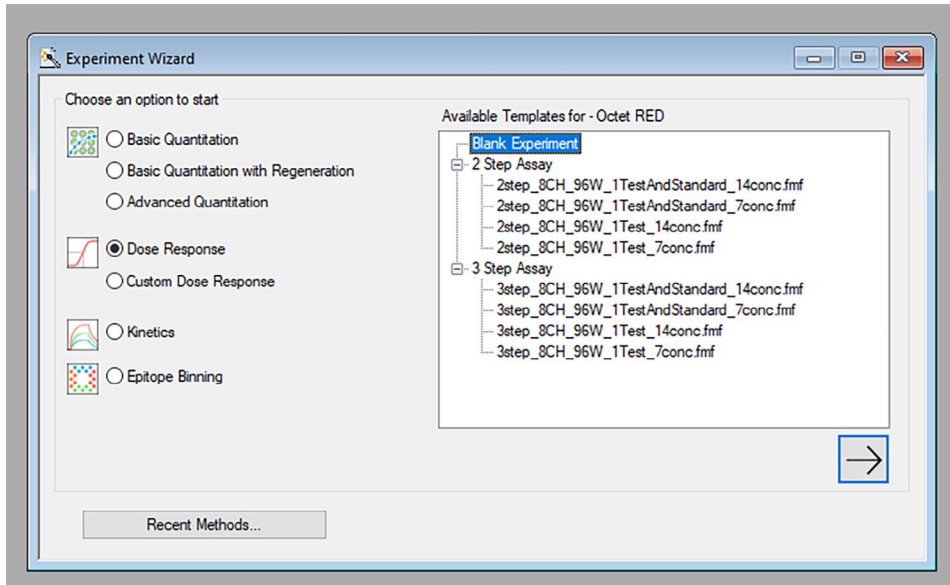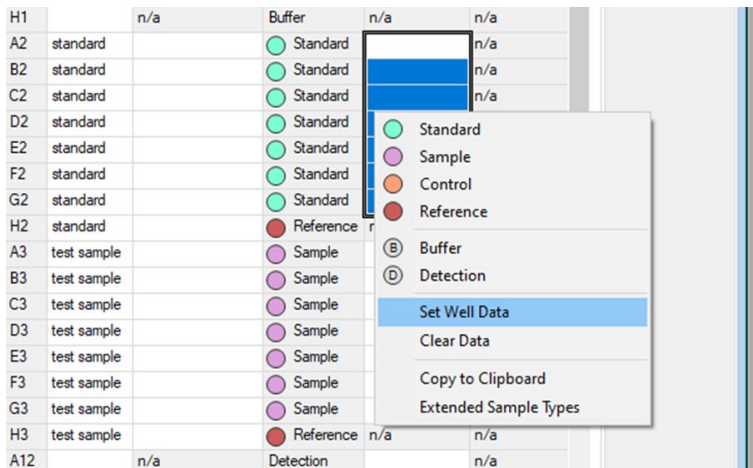
Figure 2

Figure 3

Data Analysis

During data analysis the Octet® Analysis Studio software automatically detects, assigns, and preprocesses the data generated using the Octet® BLI discovery software and displays this i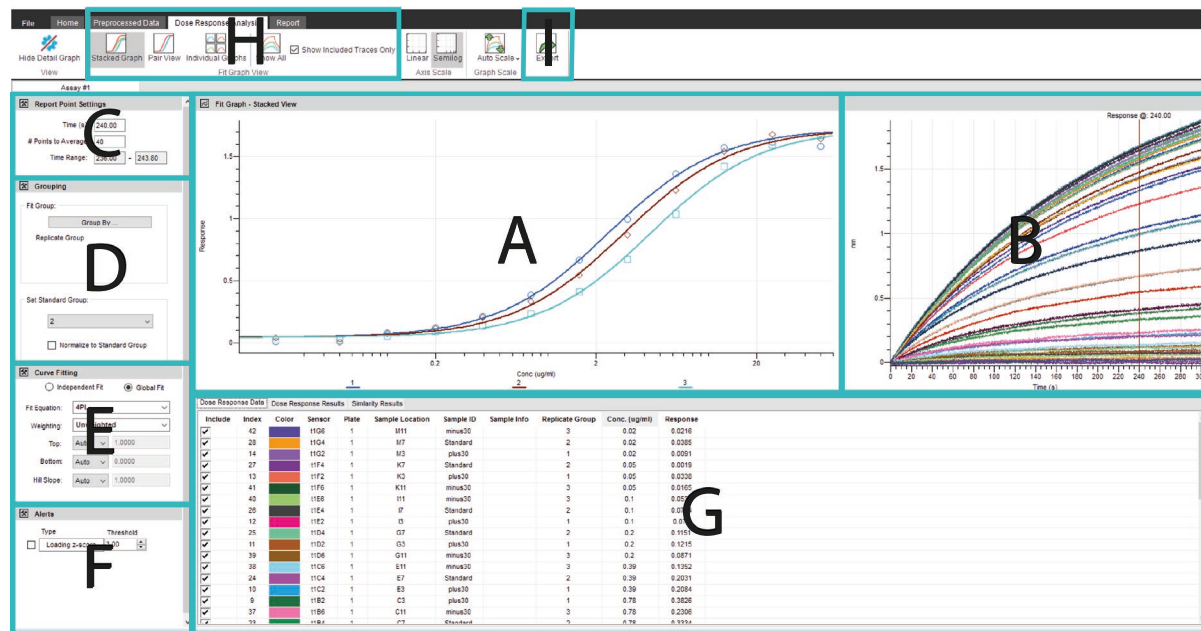n the Preprocessed Data tab. The Pre-processed Data tab is a good time to perform a visual quality control check to check for any issues during the assay, such as if one channel has not loaded as well as the others and can be excluded.

Dose Response Analysis

The Dose Response Analysis tab (Figure 4) allows the user to have access to a range of powerful data fitting options that are updated in real-time and shown in the Fit Graph window (Figure 4A).

Figure 4

Report points for use can easily be set using the sample response window (Figure 4B) by placing the blue vertical line where desired or a specific report point time and number of points to be averaged can be set in the Report Point Settings (Figure 4C). It is also possible to normalize to the standard group across samples.

Thanks to fully customizable two- and three-step basic assay templates, it is possible to generate multiple data sets of test materials and standards. The grouping window (Figure 4D) allows you to group the data in a number of different ways including the default averaged data sets or the presentation of individual replicate curves where desired. An important part of a relative potency assay is determining the similarity (EC_{50}) of the test material(s) versus a known standard, and this can easily be set using the 'Set Standard Group' option. If set, normalization of the test materials to the standard can be performed using the normalize to Standard Group option (Figure 4D), which takes the minimum and maximum response from the standard curve and applies normalization to all the test material curves. The scale in the Fit Graph window (Figure 4A) changes accordingly from nm response to a scale of 0–100.

Of critical importance in determining similarity (EC_{50}) is the model used for fitting the standard and test material(s). Octet® BLI analysis software offers a range of fit parameters including independent and global fits, which applies the same parameter fit to all the groups but each test material has its own EC_{50} value. Data can be fitted to 3, 4, and 5 parameter logistic (PL) fit equations along with linear and semilog line fits. Pre- and post-transition baselines play a key role in determining accurate relative potency and therefore, if these parameters are not well defined, a top and/or bottom value can be assigned for all curves prior to fitting (Figure 4E).

Real-time monitoring of load steps using label-free assays is a major advantage over endpoint-based assays and allows the user to assess whether observed differences are due to loading issues or actual variability in the assessed samples. Responses that propagate due to differences in the loading level are often not linear and therefore, the Z-score function allows the user to set an acceptance criterion for the loading tolerated threshold. When checked, loading Z-score (Figure 4F) adds a data column to the Dose Response Data (Figure 4G), which shows the variability in loading. Where the loading Z-score is above the user-defined threshold, the data set is flagged for further investigation.

Analyzing Similarity

Fitted data can be viewed at all times in the Fit Graph window (Figure 4A) and similarity results viewed in the data table Similarity Results tab (Figure 4G), where each test material's fit is compared to the standard and their similarity based on EC_{50} is shown.
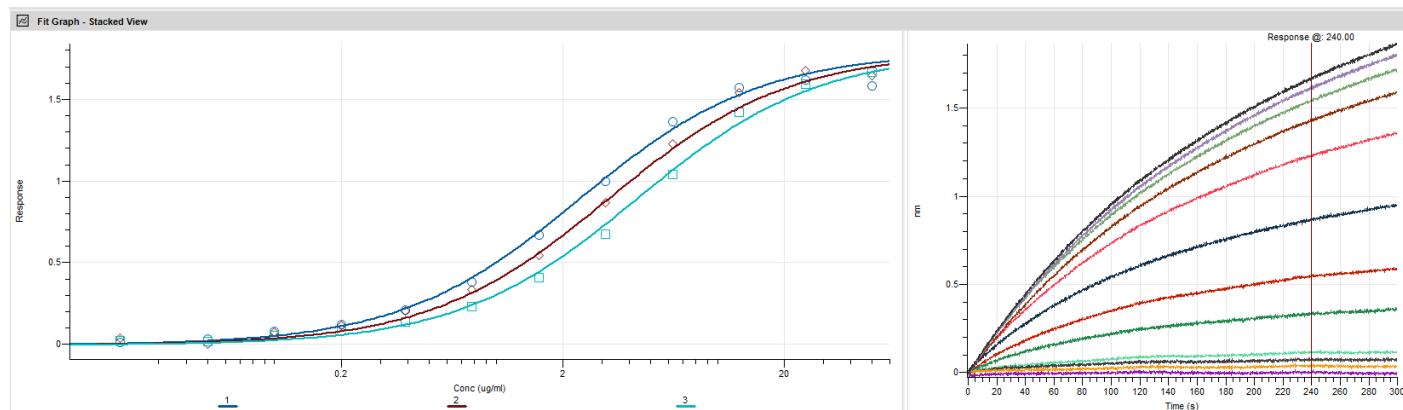
A range of data views is available using the 'Fit Graph View' box (Figure 4H) where test material(s) and standard data can be shown stacked on a single graph or in pairs or individual graphs. Stacked graph view is the default view, which shows the standard and all test materials on the same chart. Once prepared, data can easily be exported in numerous ways (Figure 4I) for further analysis or custom reports can be made in the report tab.

Assessing the Relative Potency of Human IgG Antibody Samples

Assessment of antigen binding for antibody development is a critical step, and the ability to accurately detect changes in binding is critical. In a setup that would be suitable for assessing two unknown antibodies against a reference standard: an Octet® R8 8-channel BLI system and AHC2 biosensors (18-5142) were used in a standard 2-step dose response assay format. A 12-point dilution series (50–0.02 µg/mL) of human IgG (hIgG) reference samples were then tested using an anti-hIgG Fab detection step (5 µg/mL). The assay was then repeated with either a plus or minus 30% hIgG concentration series (65–0.03 µg/mL and 35–0.01 µg/mL, respectively), and the data analyzed using the Octet® Analysis Studio Dose Response module (Figure 5). All curves were assessed using a 4 PL equation and normalized against the reference standard series. Each concentration series showed an R^2 value >0.99 for each data set, and comparison of the EC_{50} ratio for each series exhibited a similarity score of 1.34 and 0.74 for the plus or minus 30% hIgG concentration series when compared to the reference standard, in good agreement with the predicted value. Combined with the Octet® Analysis Studio Dose Response module, the above assay format allows users to accurately assess antigen binding of multiple antibodies with good accuracy.

Figure 5

Conclusions

The addition of Dose Response Analysis to the Octet® BLI Analysis Studio software allows users to rapidly assess the EC_{50} of samples and determine similarity compared to a standard data set. The Octet® BLI Analysis Studio software is fully compatible with data generated in previous versions of the software allowing rapid assessment of both previously generated and new data without the need to export data to third-party software.

Real-time, label-free relative potency assays are now simpler than ever on the Octet® BLI systems meaning that laborious endpoint assays with long wash and incubation steps and high hands-on time can become a thing of the past. Octet® BLI relative potency assays allow the user to observe the loading levels of their proteins and assess the data accordingly, and unlike endpoint assays, kinetics of the interactions can also be extrapolated, even for low-affinity molecules.

References

1. Bradbury A, Trinklein N, Thie H, *et al.* When Monoclonal Antibodies Are Not Monospecific: Hybridomas Frequently Express Additional Functional Variable Regions. *MAbs.* 2018;10(4):539–546.
2. <1032> Design and Development of Biological Assays. doi:10.31003/uspnf_m1354_01_01