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Application Note

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Designing and Measuring Drug Potency Using the Octet® BLI Ligand Binding Methods

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Abstract

In this application note, we describe the use of the Octet® Biolayer Interferometry (BLI) platform to develop methods for the determination of EC_{50} as a measure of antibody-based drug potency. Two methods; a 2-step dose response and a 3-step dose response designs are described with case studies exemplifying each of the methods given. Case studies exhibit the use of either antibody-based biosensors such as anti-human capture (AHC2) biosensors or Streptavidin biosensors as appropriate for use in either method format. The Octet® software (with features for comparison of EC_{50} of multiple drug molecules) is used to extract important parameters that can be further used to assess similarity between different molecules.

Introduction

Potency monitoring is a regulatory requirement for therapeutics drug product release for the purposes of ensuring clinical effectiveness of the therapeutic throughout its stipulated life cycle. A potency assay is a quantitative measure of biological activity of the product; it must represent the intended biological effect that can be related to clinical response where possible. Potency assays help provide assurance of the quality and consistency of the product. Reproducible and accurate assays for measuring drug potency are the cornerstone of quality control of manufactured products. Increasing precision of analysis leads to a better ability to determine lot to lot variability in the manufactured product, assess the impact of process changes on drug quality and assess the product's stability over time. Methods to monitor potency therefore need to be stability indicating meaning that analytical instruments that can be used for stability indicating methods can be used to monitor potency through the interaction between a target and the therapeutic(s) in a dose response manner as a function of time. Potency measurements from these methods should be used to demonstrate that only product lots that meet defined specifications or acceptance criteria are used during all phases of clinical investigation and after the therapeutics approval[1,2]. Two critical parameters; Effective Concentration (EC_{50}) and Inhibitory Concentration (IC_{50}) can be used to evaluate efficacy and potency.

Due to potential variability in biological assays, relative potency (RP), a measurement of activity of a test material relative to a known standard is more suitable. It assumes that the reference and test material are biologically similar and that the test material behaves like a concentration or dilution of the standard[3]. While potency or relative potency can be measured using techniques such as ELISA, real-time Biolayer Interferometry can provide several benefits over ELISA. The assay is direct and label-free and can substantially reduce assay development time compared to endpoint assays.

Designing a potency assay on the Octet® BLI system

Octet® BLI systems utilize Biolayer Interferometry technology for detection of the interactions of biological molecules. Propriety biosensors coated with biological molecules such as Protein A, anti-human or anti-murine antibodies, Streptavidin and other coatings can be used to immobilize ligands that are in turn used to bind target molecules, resulting into response signals that are analyzed for molecular interactions.

A complete coverage of the dose response curve relationship is essential for reporting EC_{50}/IC_{50} and ensuring the Logistic Regression accuracy of the curves. Ideally, a sigmoidal curve with at least 2 concentrations points at the linear phase should be established. It is also recommended to have at least 2-3 replicates for each sample concentration. A typical sample concentration dilution scheme starts from a high concentration with the expectation of response signal-saturation (EC_{50}) or response signal-suppression (IC_{50}) to multiple serial-diluted concentrations. It may be necessary to test a 3-fold or 2-fold serial dilution to ascertain the requirement of data points at the low and high asymptotic phases and the linear phase of the curve.

For example (Figure 1), given an antibody with a signal-suppressed concentration of 100 µg/mL against the target molecule, a 5-fold dilution is performed making 100, 20, 4, 0.8, 0.16, 0.032, 0.0064 µg/mL + 0 concentration buffer as reference (n=2) is sufficient to capture the full EC_{50} dose-response relationship. However, without prior knowledge, quick scouting experiments should be performed to determine the signal-saturated (EC_{50}) (or signal-suppressed concentration for IC_{50}), as well as the dilution factor.

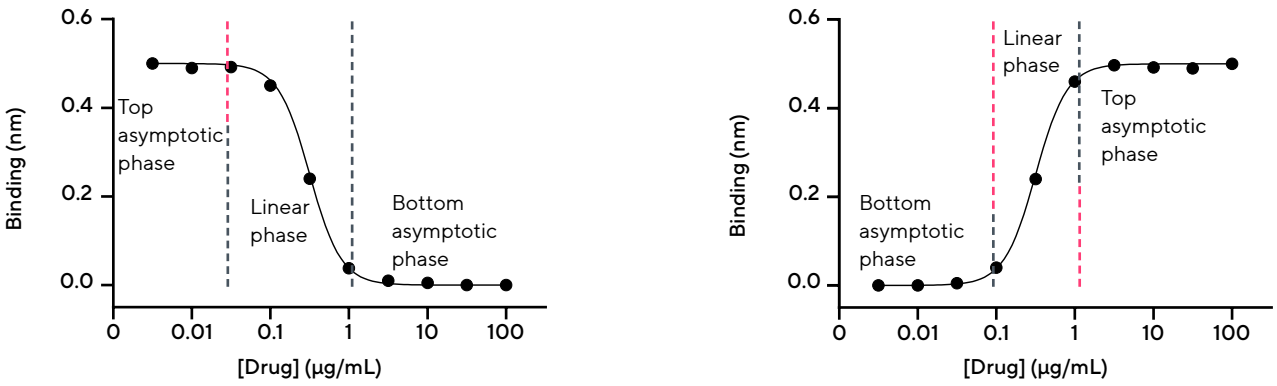

Figure 1: Representation showing a minimum Dose-response (IC_{50} (A)) and (EC_{50} (B)) plots. Black dots denote experimental data. Black curve denotes 4-parameter Logistic (4PL) regression curve (n=2).

The Octet® BLI system software (v13) for example, enables robust methods development for high-throughput screening. Typical assay formats for performing dose-response (DR) assays are, 2-step and 3-step formats (Figure 2) mostly, depending on the need to capture functional binding properties in the assay.

2-Step and 3-Step Dose-response Assays

A 2-step DR assay starts with immobilization of a ligand to the BLI biosensors, followed by association of various concentrations of analyte to the immobilized ligand for detecting various levels of response while a 3- step dose response assay is used when there are binding partners other than the analyte. The 3-step DR assay is recommended for use when there's a physiologically relevant biomolecular interaction where the activity is modulated by a third binding partner[1]. The third binding partner is used as the detecting molecule.

In this document, we describe different scenarios where the Octet® BLI platform is used as a suitable tool for DR measurement as a parameter for the assessment of potential drug/reagent quality differences. We compare the DR curves of +/- 30% of change in hIgG concentrations relative to the nominal concentration in a 2-step DR assay where human antibody (hIgG) is first immobilized onto Octet® AHC2 Biosensors followed by the interaction of Fab anti-hIgG to compare potency. Next, we subject the hIgG samples to heat stress and measure potency as a function of sample stability.

To illustrate the use of the Octet® BLI system in a 3-step DR assay format, we use biotinylated TNFα, TNFα antibodies and CD64 as binding partners.

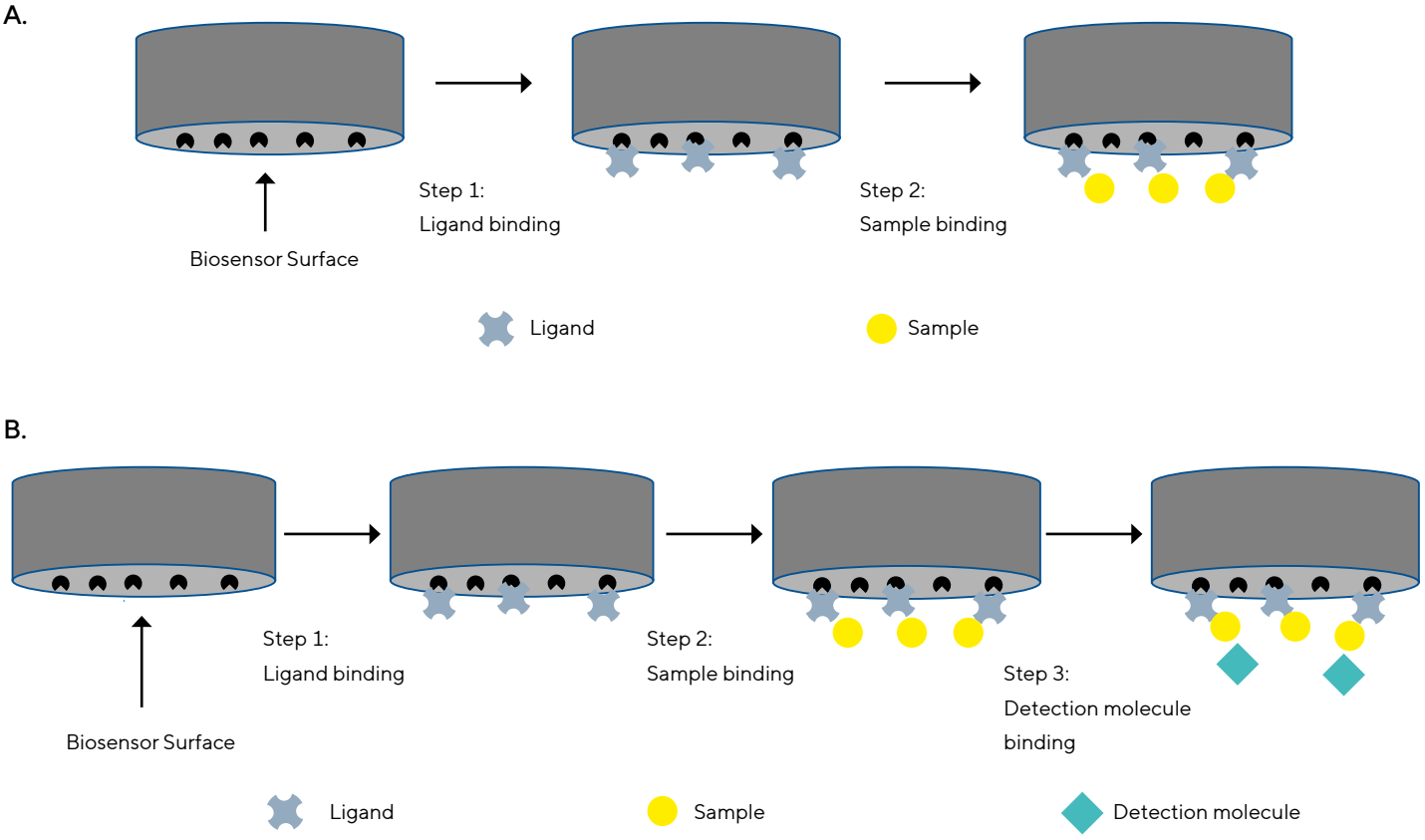

Figure 2: A schematic of 2-step (A) and 3-step (B) assay designs on the Octet® BLI system. While the 2-step assay design is used to directly measure and analyze data from a dose response of the sample directly; a 3-step assay design requires a subsequent detection step where a constant concentration of the detector molecule is used to generate binding response. The binding response is then correlated to the sample concentrations in the previous step.

Case study 1: EC_{50} as a function of varying antibody nominal concentration

In case study 1: We sought to illustrate the functional assessment capability of a BLI-based DR assay pipeline by performing a 2-step dose-response analysis of human antibody (hIgG) samples captured to Octet® AHC2 Biosensors followed by the interaction of Fab anti-hIgG first to showcase dose response differences as a function of % nominal concentrations and second to compare potency and efficacy changes that may be induced by changes in sample integrity. EC_{50} analysis and other assay parameters were used to determine potency.

Materials and Equipment:

- Octet® R8, RH16, or RH96 BLI systems
- Octet® AHC2 Biosensors (PN 18-0057)
- 10X Kinetics Buffer (PN 18-1105)

Sample: hIgG (Lampire PN 7799999)
Detection reagent: Fab anti-hIgG (Rockland PN 39353)

Assay steps were defined using the modifiable assay method settings (below) from the Octet® BLI Discovery Software.

Assay Settings
Assay: **Dose Response**
Standard Assay
Single analyte

Modify

	Time	Shake speed (rpm)
Buffer	180	1000
Sample	300	1000
Buffer	240	1000
Detection	300	1000

Human IgG Samples were prepared in 1X KB at concentrations ranging from 200 – 0.02 µg/mL (14 data points). Samples were prepared to 130%, 100% and 70% of the nominal concentration and were used as ligand. For each % nominal concentration a set of Anti-human capture (AHC2) biosensors were used to capture the antibody sample followed by the binding of Fab-anti-hIgG (5 µg/mL) as the detection reagent (Figure 3A). The detection step of the assay was later aligned (Figure 3B) in the Octet® Analysis Studio Software and was used to plot response as a function of sample concentration for the three different nominal concentration samples. As expected, higher concentrations (130 % of nominal concentration) shifted the dose response curves to the left, while the lower (70 % of nominal concentration) shifted the dose response curves to the right. The Octet® Analysis Studio Software v13 was used to determine EC_{50} values and other critical assay parameters (Table 1A).

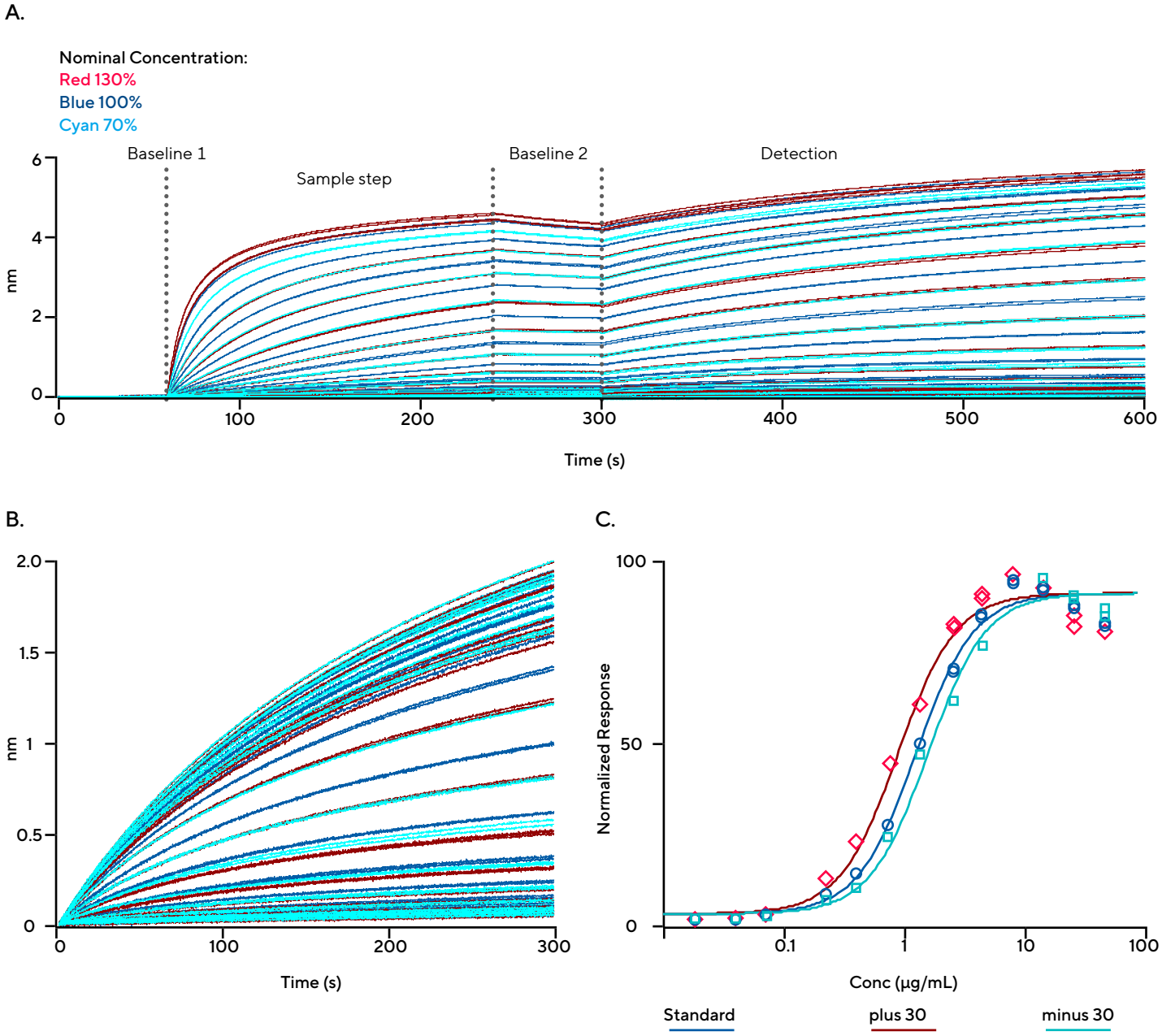

Figure 3: (A) 2-step assay setup with sensorgrams displaying BLI responses at various steps of the nanometer shift responses of the potency assay. A baseline is first established with Octet® AHC2 Biosensors in 1X KB buffer for 180s followed by 300s association with three series dilutions of starting concentrations corresponding to 130% (Red), 100% (Blue) and 70% (Cyan) of nominal hIgG sample concentration, respectively. Next, AHC2 sensors moved to 2nd 1X KB buffer for 240s baseline, followed by 300s detection steps in 5 µg/mL Fab anti-hIgG. An identical assay was repeated with fresh sensors for a technical duplicate. (B) Response values of detection step aligned to initial detection time. Responses at 300s of the detection step were reported. (C) Differential detection responses of 130% (Red), 100% (Blue) and 70% (Cyan) of nominal hIgG sample concentration demonstrating 2-step EC_{50} Dose-response assay workflow. The two highest concentration 200 and 100 µg/mL datapoints were removed to eliminate the hook effect that could decrease the model fitting accuracy.

A different set of human IgG samples were used to demonstrate stability indication of the method through a deliberate inactivation of the samples. In this study, one set of 100 % nominal concentration of the samples were heat stressed for 5 minutes on a heating block at 95°C. Dose response analysis was performed similar to the previous assay (Figure 4A).

Dual EC_{50} curves showed lower maximal stable response and overall curve shift to the right for heat stressed hIgG interacting with Fab anti-hIgG, relative to intact hIgG. The result suggests 5 min 95°C sufficiently lowered the maximal effect and attenuated the potency of the hIgG to Fab anti-hIgG. Responses at 300s of the detection step were reported and were fitted in the Octet® Analysis Studio Software to extract EC_{50} values and other parameters (Table 1B).

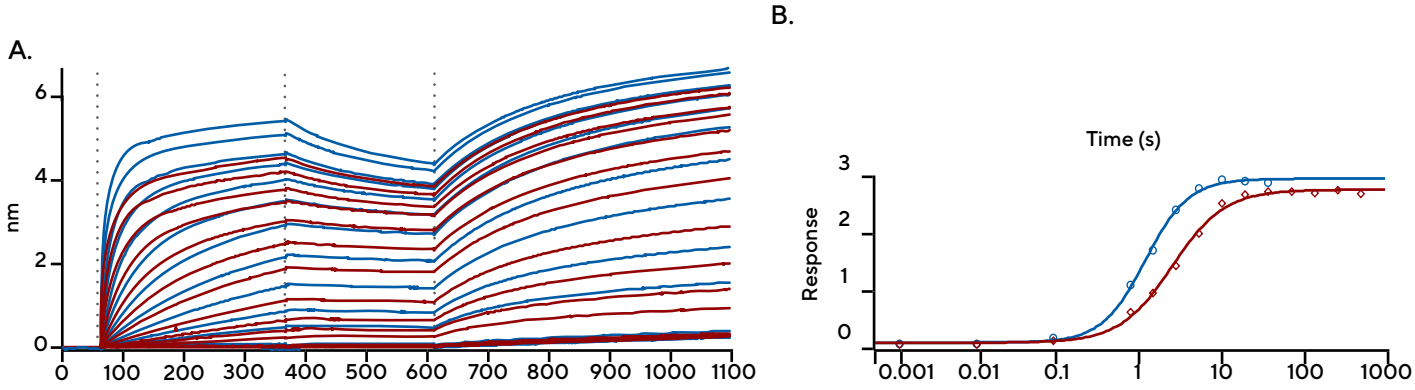

Figure 4: (A) An overlay of sensorgrams for heat-stressed and non-heat stressed human IgG samples in a 2-step dose response potency assay. A baseline is first established with Octet® AHC2 Biosensors in 1X KB buffer for 180s followed by 300s association with three series dilutions of starting concentrations using 100% nominal sample concentrations. (B) Sample detection step response analyzed at 300s (the heat stressed samples response shifts to the right indicating lower stability when inactivated (brown curve).

A.

Parameter	Standard	130%	70%
EC_{50} (µg/mL)	3.459	2.66	4.66
EC_{50} Relative to standard	100%	77%	135%
Hill Slope	1.3296	1.3296	1.3296
Bottom	1.0369	1.0369	1.0369
Top	100.6103	100.6103	100.6103
R^2	0.9968	0.9941	0.9959
Global R^2	0.9956	0.9956	0.9956
EC_{50} 95% CI	3.27 to 3.66	2.51 to 2.82	4.4 to 4.93
Hill Slope 95% CI	1.25 to 1.41	1.25 to 1.41	1.25 to 1.41
Top 95% CI	99 to 102	99 to 102	99 to 102
Bottom 95% CI	-0.11 to 2.18	-0.11 to 2.18	-0.11 to 2.18

B.

Parameter	Intact hIgG	Heat stressed hIgG
EC_{50} (µg/mL)	1.97	5.21
Hill Slope	1.58	1.33
Bottom	0.035	0.018
Top	2.47	2.09
EC_{50} 95% CI	1.78 to 2.18	4.77 to 5.70
Hill Slope 95% CI	1.34 to 1.83	1.19 to 1.47
Top 95% CI	2.40 to 2.55	2.05 to 2.13
Bottom 95% CI	-0.03 to 0.1	-0.028 to 0.063
R^2	0..997	0.998
Chi^2	0.031	0.023

C.

Parameter	Intact hIgG	Heat stressed hIgG
EC_{50} (µg/mL)	1	2.671
Hill Slope	1	0.89416
Bottom	1	0.5053
Top	1	0.8442

Table 1 : A Summary of Octet® Analysis Studio Software v13 fitted parameters for Dose-response of Fab anti-hIgG to hIgG with +/-30% change in nominal concentration (n=2). Global 5PL fitting showing EC_{50} values negatively correlated with change in nominal concentration. Average and standard deviation were calculated from n=2 biological replicates. B: Data EC_{50} (µg/mL) reveals that a 5 min 95°C treatment of the hIgG sample leads to lower potency (larger EC_{50}) and less maximal effect, relative to intact hIgG. Response values were grouped by treatment and subjected to 4PL unweighted regression. C: Further Similarity analysis Similarity of relative potency between heat stressed and intact hIgG confirm negative effect of 5 min 95°C treatment on hIgG antibody efficacy and potency.

Case study 2: EC_{50} Analysis for reagent vendor lot quality assessment

A potential use of the Octet® BLI system in reagent characterization and potency assessment is to compare and qualify reagent lots from different vendors or from different manufacturing lots for product development. In a study to showcase the 3-step DR assay method for potency testing, we used the TNFα model to compare the potency of two anti-hTNFα lots from two different vendors. Therapeutic anti-TNFα antibodies function by blocking sites on TNFα hence preventing binding to their receptors. Fc-receptor binding can therefore be used to assess anti-TNFα potency

Materials and Equipment:

- Octet® R8, RH16, or RH96 BLI systems
- Octet® Streptavidin (SA) Biosensors (PN 18-5019)
- 10X Kinetics Buffer (P/N 18-1105)
- **Loading:** Bh-TNFa (Sino Biological PN 10602-HNAE-B)

Sample 1: anti-hTNFα (Vendor A)
Sample 2: anti-hTNFα (Vendor B)

Assay Step	Step Time (s)
Loading	60
Baseline 2	180
Sample	600
Baseline 3	600
Detection	600

The experimental setup was such that biotinylated TNFα was first immobilized onto Streptavidin biosensors followed by the binding of the different vendor sourced antibodies; finally, CD64 (FcγRI) was used at a fixed concentration as the detection reagent. The antibody samples were tested at a range of 200 – 0.024 µg/mL (a total of 14 data points at a two-fold serial dilution was used).

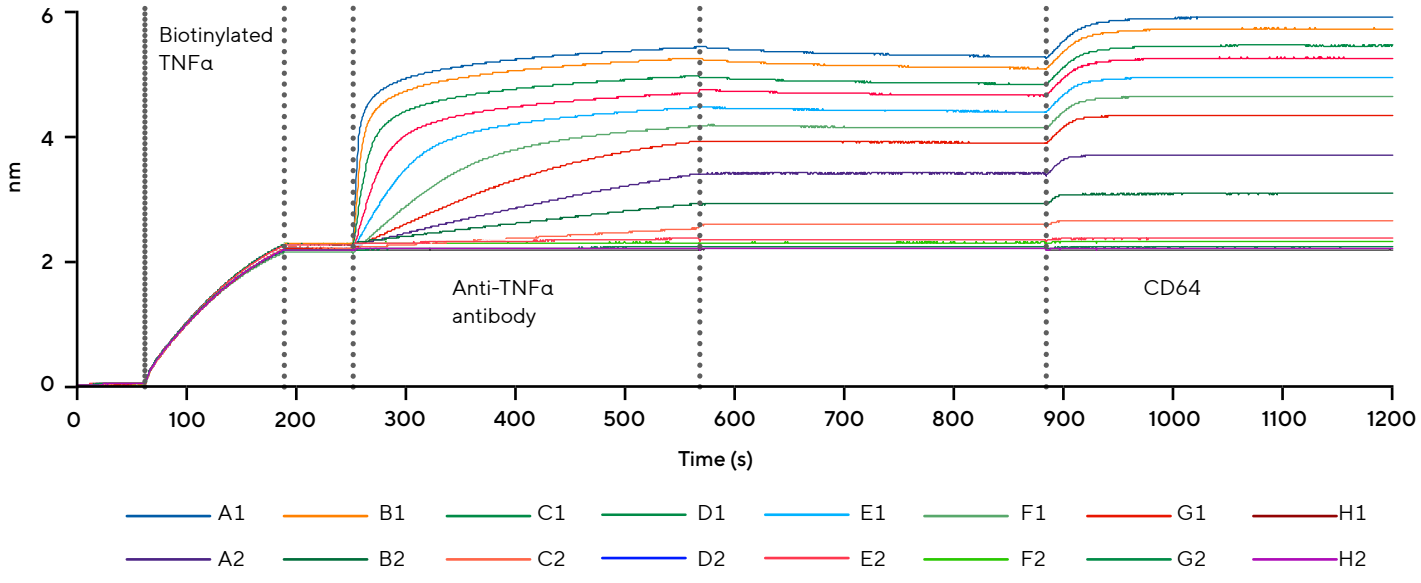

Figure 5: A 3-step DR Sensorgrams of therapeutic TNFα antibody from different vendors quality check through EC_{50} analysis displaying responses at various steps of the assay (curves shown for one vendor lot). All reagents were made in 10X KB buffer. The sample (antibody binding step used samples in the range 200 – 0.024 µg/mL while the detection step (CD64) was monitored using a fixed concentration of 1 µg/mL.

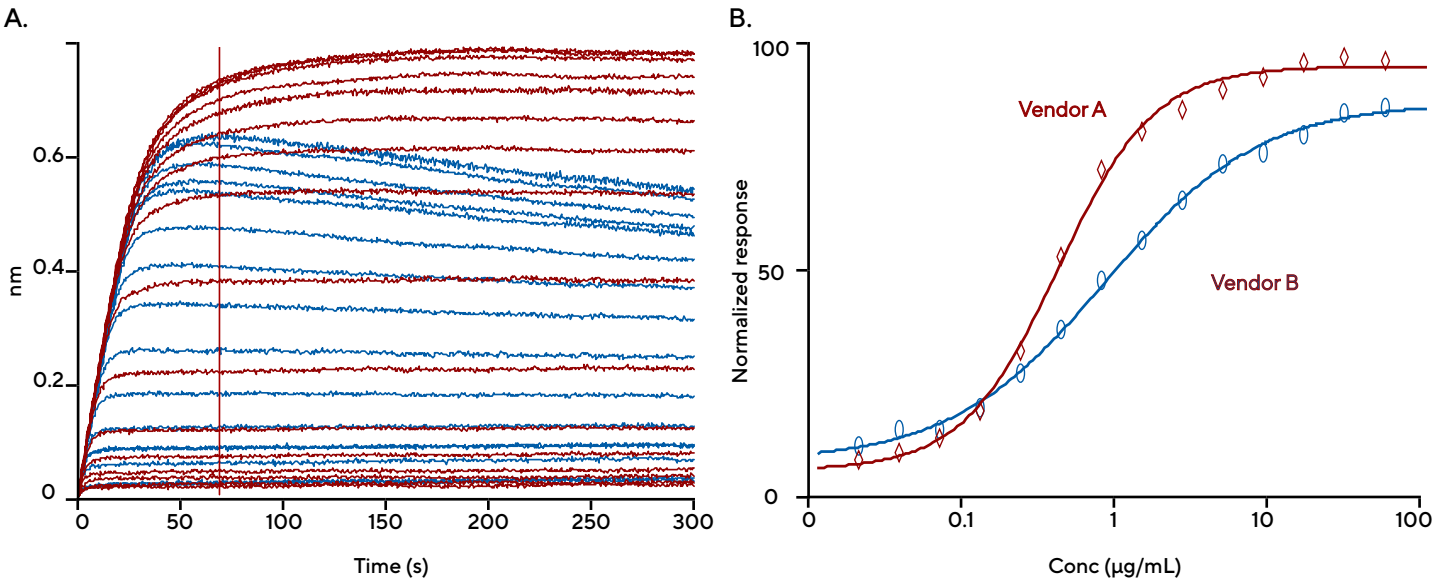

Figure 6: Duplicates for each vendor sample's DR of the detection step (A) at a response time of 60s was plotted as a function of vendor sample concentration. Higher and more stable responses (B) were observed with vendor A samples indicating a more potent product from Vendor A.

A further analysis of the Octet® software fitted data (Table 2) indicates that Vendor A product is almost 2X more potent than the product from Vendor B

Vendor	EC_{50} (µg/mL)	EC_{50} relative to Vendor A	Hill Slope	Bottom	Top	R^2	EC_{50} 95% CI	Hill Slope 95% CI	Top 95% CI	Bottom 95% CI
A	0.72	100%	1.29	1.21	96.31	1.00	0.64 to 0.801	1.123 to 1.1457	94.364 to 98.249	-2.099 to 4.518
B	1.25	174%	0.67	-3.72	89.12	1.00	1.062 to 1.473	0.587 to 0.751	86.306 to 91.929	-7.827 to 0.385

Table 2: Summary of Octet® Analysis Studio Software v13 3-step Dose-response fitted parameters (n=2) for two different vendor lots of an anti-TNFα antibody.

Summary

Potency is a critical quality attribute for therapeutic drugs that need to be assessed and maintained throughout the lifecycle of the drug. While actual potency needs to be assessed using cell-based assays that better mimic the clinical environment for the therapeutic drug, cell-based assays are often more variable and are a lot more challenging to control than ligand-based assays. Ligand based assays can therefore be used as platforms for surrogate potency assessment methods especially earlier on in the drug product development cycle. In addition, the assessment product potency and other binding parameters can be useful tools for overall quality checks between multiple vendor lots and serve as a quick tool for reagent characterization and selection for downstream product development. In this application note, we have demonstrated two potential methods for the rapid assessment of potency; one a 2-step dose response method where samples are directly immobilized to the biosensor surface followed by a detection step and two, a 3-step method that assumes physiological relevance of multi-binding partners such as in a blocking or neutralization assay and where one of the binding partners is used as a detection reagent. In both assays, multiple relevant parameters include EC_{50} and a Hill-slope can be extracted and used to determine the potency and efficacy of the drug. Data analysis is further simplified by new capabilities in the Octet® Analysis Studio Software v13.

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