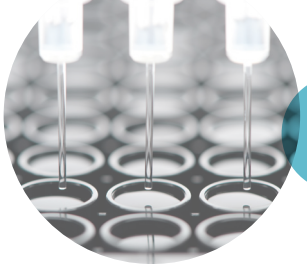
FORTÉBIO



Quality control of biotherapeutics using Octet systems

Bio-Layer Interferometry (BLI) has been rapidly adopted as an important analytical tool in laboratories that work with biological molecules, either as drug products, vaccines, or diagnostic reagents. Widely-used and accepted in research and assay development, BLI assays are now also utilized for quality control (QC) in biologics development workflows for ligand binding and product quantitation during lot release and in-process testing.

Octet® systems combine real-time binding analysis with a simple Dip and Read™ approach for rapid assay and method development. For example, the 8-channel Octet RED96e system quantitates 96 mAb samples in only 30 minutes, compared to >6 hours by ELISA[1] or as long as 10 hours via HPLC, resulting in significant project cost and time savings. Optimizing a generic potency assay takes approximately half the time on Octet systems as it would with SPR or on manual ELISA.

Octet systems also offer several other advantages over comparative analytical technologies. The microplate-based sample format is ideal for Design of Experiments (DoEs), enabling rapid, high-throughput method optimization. Disposable, ready-to-use or customizable biosensors are simply dipped into samples for analysis – no priming needed. This lack of microfluidics results in a more robust and flexible platform that can also accommodate direct analysis of crude samples.

Is the Octet platform GxP ready?

All Octet instrument models are GxP ready. ForteBio offers GxP users an assortment of products and services that ensure full confidence in data integrity. They include:

- 21 CFR Part 11-compliant software that can be used on all Octet models including Octet K2, RED96e, RED384, QK^{e} and HTX
- Instrument Installation and Operational Qualification (IQOQ) Kits
- Performance Qualification (PQ) Kit validated for the Octet RED96e instrument
- High Precision Streptavidin 2.0 biosensors (SAX2) qualified for seamless use across multiple lots of biosensors
- Support service for customer-run software validation

Is the Octet platform recommended for use in regulated environments?

Regulatory bodies such as the FDA, EMEA, NMPA and others do not typically recommend or refer to specific product, brand, or vendors in their guidance on analytical technologies. While there may be references to certain technologies in some USP chapters, these are references based on general concepts around the application in question. Label-free methods such as BLI and SPR are simplified immunoassays based on the ELISA-type binding assay principle, so their use for measurement of potency or product quantity is based on decades of established and accepted measurement principles. Binding kinetics constant measurements using label-free methods are also well established, and BLI technology has been demonstrated through use in thousands of laboratories world-wide and in peer-reviewed publications. In fact, best practice in the drug development industry employs orthogonal or alternative platforms that are fit for purpose. The key factor here is that the sponsor sets appropriate acceptance criteria and that the technology in use enables the development of suitable methods that are robust and meet the criteria for quality control for the specific product.

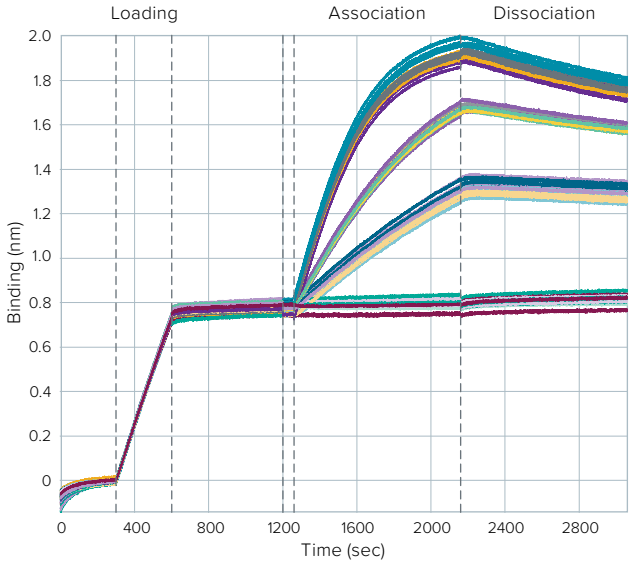
Which Octet biosensors are suitable for QC methods?

The selection of biosensor surface chemistry depends on the application. All Octet biosensors can be used to develop QC methods. However, during assay method development, repeatability, intermediate precision and reproducibility studies that include biosensor lot to lot assessment should be done to determine assay robustness in line with recommendations from the relevant regulatory bodies. High Precision Streptavidin 2.0 biosensors (SAX2) (Figure 1), intended for use with biotinylated ligands, have been developed to ensure minimal lot-to-lot variations in ligand immobilization. This biosensor is recommended for use with any assay where high ligand immobilization reproducibility is critical. The SAX2 biosensor is suitable for both ligand binding kinetics assays and for custom quantitation assays (through pre-coating of a capture ligand on the biosensor).

What if I have a different Octet model in development than the one in QC – is Octet data comparable?

Analytical method changes are a normal process in the product life cycle and can be triggered by a variety of factors such as the need to better understand product quality or to replace legacy methods, and other reasons. In any case, regulatory bodies require that bridging studies be done when a method change is necessitated and that the new method be validated. Although bridging studies are a requirement, the data obtained on any Octet system – from the Octet HTX to the RED384 or the 8-channel RED96e – should be comparable so long as the acquisition parameters are consistent across the experiments.

A



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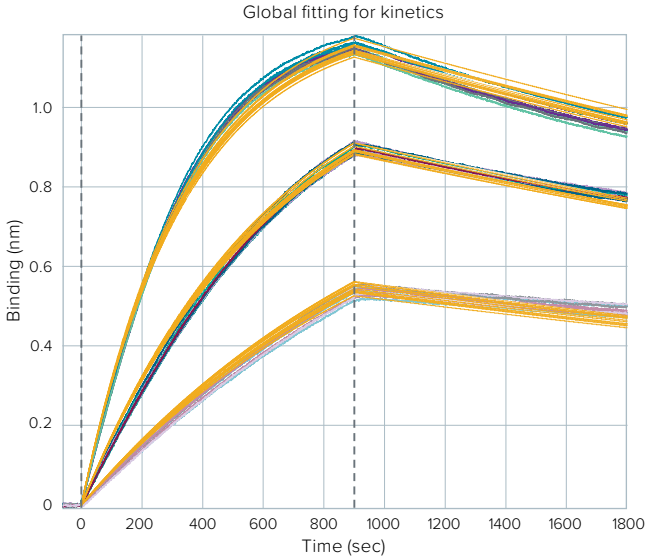

Figure 1: (A) Kinetic analysis of the interaction between a ligand biotin-Fcγ Receptor (~55 kDa) and analyte hIgG (150 kDa) with SAX2 biosensor, overlaying three lots of SAX2 and raw data aligned at the ligand loading step. (B) Data was processed and curve fitted using a 1:1 binding model. Yellow lines represent fitted curves; other colors represent raw curves. See the SAX2 biosensor datasheet for ligand loading specifications.

Figure 2 and Tables 1 and 2 show exemplary kinetics characterization data for PSA antigen binding to its antibody as derived from the three different Octet models. The data suggests insignificant differences in response signals for both ligand immobilization and antigen binding, resulting in very low variability in the extracted affinity constants. This data demonstrates that Octet systems deliver consistent performance across the platform, regardless if researchers are using multiple, same-model instruments, or different Octet instruments.

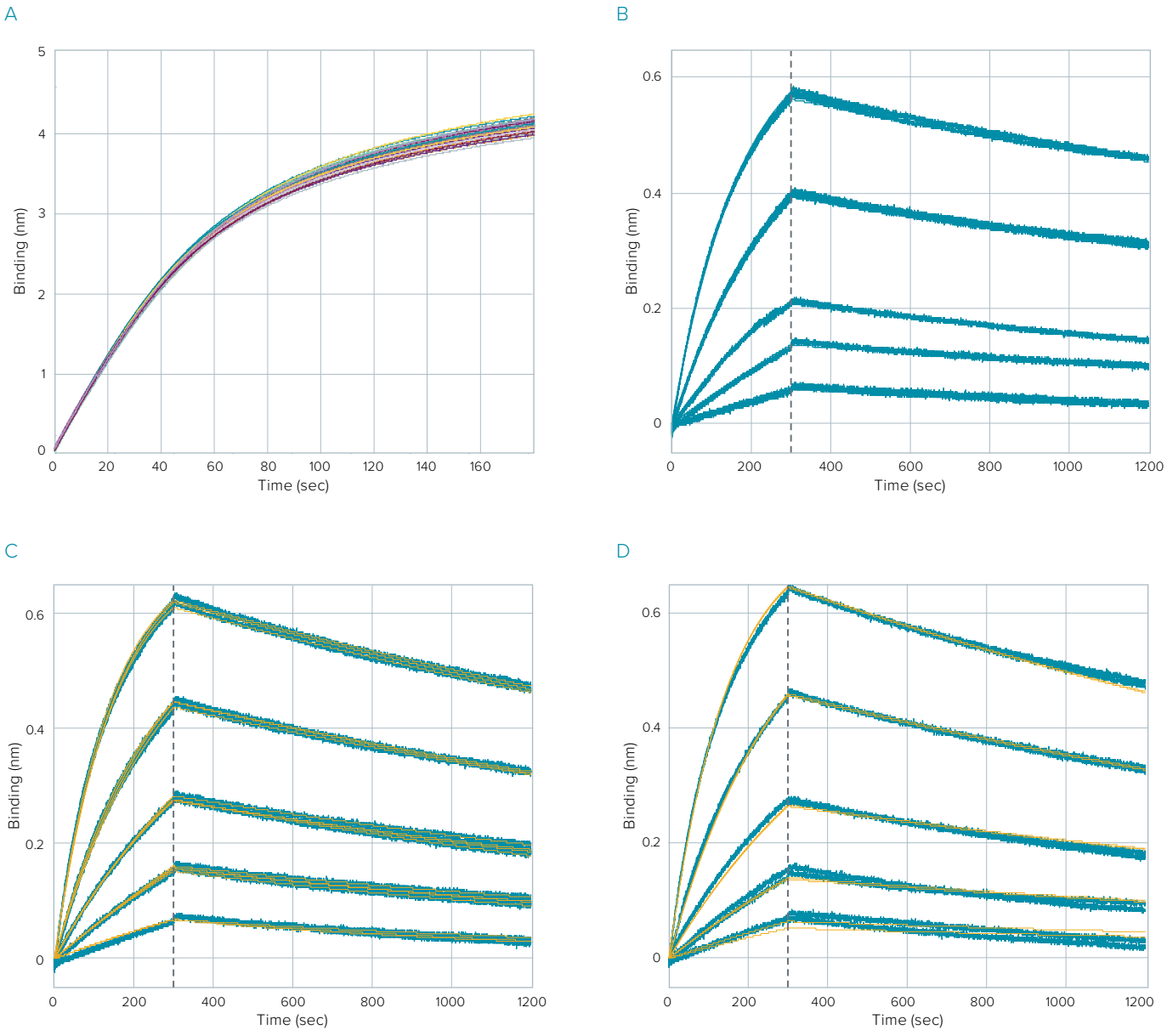

Figure 2: Octet comparability kinetics studies showing side by side kinetics data for biotinylated anti-PSA antibody immobilized on two different lots of SAX2 biosensors binding to PSA antigen. (A) An overlay of the immobilization data from three instruments where PSA antigen was used at 200 nM, 100 nM, 50 nM, 25 nM and 12.5 nM using the Octet RED96e (B), Octet RED384 (C) and Octet HTX (D) systems. Data was acquired in duplicates for each biosensor lot. Only one lot was used on the Octet HTX system. Experiments were run at 25°C using the 8-channel read head mode for all three Octet models.

Model	Mean response (nm)	Range, nm (high-low)	% CV
RED96e	1.50	1.59–1.42	3.16
RED384	1.67	1.73–1.61	2.04
HTX	1.68	1.74 –1.64	1.78

Table 1: Comparison of biotinylated anti-PSA ligand response on different Octet models. Data was obtained from two lots of SAX2 biosensors in duplicate (see Figure 2A). Loading response is reported at 150 seconds.

Octet model	Biosensor lot 1 K_D (M)	Biosensor lot 2; K_D (M)	Mean, K_D (M)	% CV
RED96e	6.91E-09	5.79E-09	6.35E-09	12.5
RED384	7.46E-09	7.13E-09	7.29E-09	3.25
HTX	7.71E-09	n.d.	7.71E-09	n.d.
Inter-instruments (mean)	7.36E-09	6.4565E-09	7.12E-09	9.79

Table 2: Octet kinetics comparability study data shows kinetics parameters for biotinylated anti-PSA antibody immobilized to two different lots of SAX2 biosensors binding to PSA antigen. Characterization was performed in duplicate for each biosensor lot and on all three Octet instruments using the 8-channel read head. Experiments were run at 25°C.

Reference

1 Biolayer Interferometry as an Alternative to HPLC for Measuring Product Concentration in Fermentation Broth, Anurag S. et al., *LCGC*, 35(12) 870–877.

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