Development of the ForteBio™ Octet System for label-free, real-time analysis of molecular interactions

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ABSTRACT

With the resurgence of interest in antibody and protein therapeutics, there is a need to accelerate their characterization and production in a simple, label-free, and information-rich format. ForteBio's Octet System, which is based on proprietary Bio-Layer Interferometry (BLI), addresses that need with label-free, real-time detection and analysis of molecular interactions.

BLI technology is employed in biosensors with a biocompatible surface optimized for protein immobilization. The Octet system uses sensitive optical detection to provide information about molecular interactions such as binding rates, affinity, and dissociation.

Octet accepts either crude or purified samples in a microplate format and presents the results in real time. The instrument can process an entire 96-well microplate in as little as 20 minutes for quantitation applications.

With a range of single-use biosensors available, Octet can be applied to a variety of research applications employing label-free detection and analysis.

Octet System

FortéBio's Octet System incorporates a dedicated instrument and a complete range of FortéBio ready-to-use biosensors for real-time, label-free detection of protein:protein interactions.

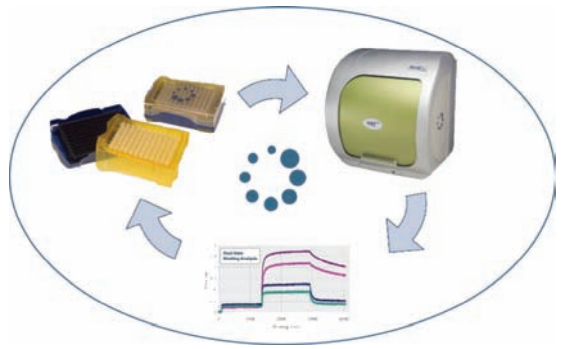

Figure 1. ForteBio's Octet System

The Octet is simple to set up and operate. Set-up consists of loading a biosensor tray and crude lysate samples presented in a standard 96-well, black polypropylene microplate. The Octet system includes user-friendly, intuitive software for operation and analysis.

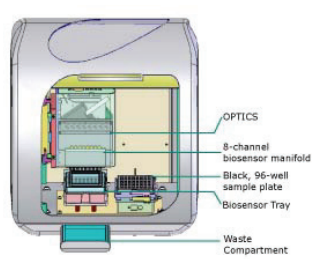

Figure 2. ForteBio's Octet Instrument

BioLayer Interferometry (BLI)

Label-free, real-time detection Technology

The Octet System is based on **B**io-**L**ayer **I**nterferometry **(BLI),** a label-free, biosensor technology for real-time measurement of protein: protein interactions. Octet biosensors are supplied with binding surfaces at the tip already derivatized and ready for use in specific applications.

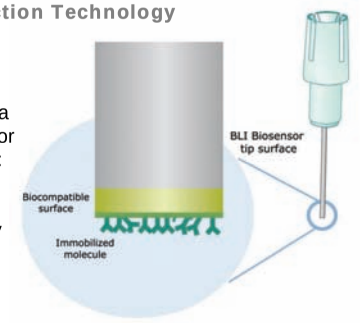

Figure 3. Octet Biosensor Surface

Surface Chemistry

A key element of FortéBio's proprietary technology is the surface binding chemistry that produces a two dimensional binding surface on the tip of an optical biosensor. The biosensor tip surface is designed to be uniform, non-denaturing and with minimal non-specific binding.

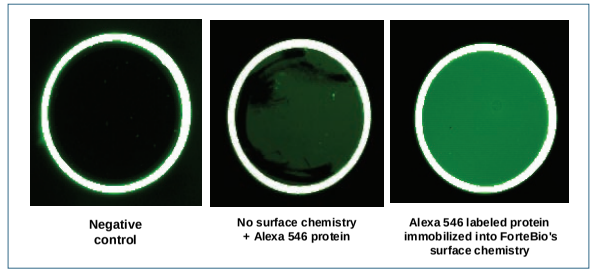

Figure 4. Octet Biosensor uniformity

To determine uniformity on the biosensor surface, Alexa 546-labeled protein was immobilized on 2 different sensors, one sensor complete with the biocompatible surface matrix and one without. Using a fluorescence scanner, protein immobilization was measured on the tip of the optical sensors. The biocompatible matrix exhibited a uniform surface over 600um in diameter. An unmodified, unlabeled sensor was scanned as a negative control.

Reproducibility

The Octet System and Biosensors produce real-time results that are highly reproducible. Here, eleven anti-Human IgG FC biosensors were assayed with10μg/ml Human IgG on 2 separate instruments and evaluated over 60 seconds. An overall precision of 6.2 % CV was obtained.

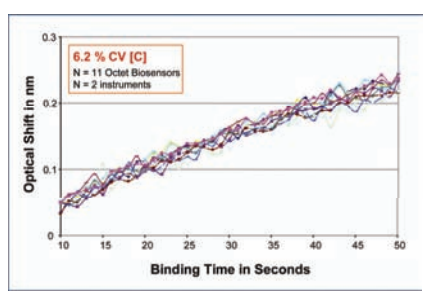

Figure 5. Initial binding kinetics at 10 μg/ml hu IgG using ForteBio anti-human IgG FC Biosensors

Measuring Protein:Protein Interactions with the Octet System

Octet provides real-time monitoring for protein: protein interactions and binding events. Any change in the number of molecules bound to the biosensor tip changes the optical path. Small changes in path length cause a shift in the interference pattern that can be measured (Δλ) in real time.

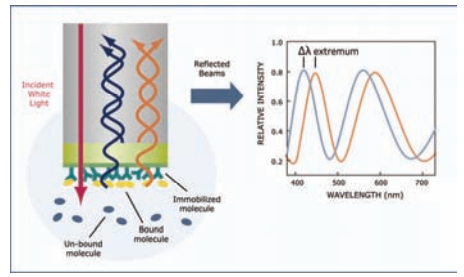

Figure 6. Bio-Layer Interferometry (BLI) Technology

Adding molecules (binding) to the biological layer shifts the wavelength peaks to the right. Removing molecules (dissociation) reduces the thickness of the layer and shifts the wavelength peaks to the left. The wavelength shift (Δλ) is a direct measure of the change in thickness (nm) of the biological layer.

Broad Matrix Compatibility

BLI technology exhibits minimal interference from biological sample media because it only detects binding at the sensor surface. To determine compatibility, 25μg/mL of hIgG was spiked into each type of media listed. Table 1 lists the percent recovery of the spiked hIgG from each sample.

		1X undiluted	1/5X in BSA Dil
Serum free	CD CHO	100	104
	CHO-S-SFMII	100	96
	CD Hybridoma	118	102
	Hybridoma-SFM	93	92
	PFHM-II	118	96
10% FBS	DMEM (w/ **Hyclone** FBS)	100	107
	IMDM (w/ Hyclone FBS)	112	108
	RPMI (w/ Hyclone FBS)	97	104
	DMEM (w/ **Cellgro** FBS)	106	97
	Overall average	105	101

Table 1. Effect of media on BLI Technology

Kinetics

With the Octet System, protein binding and dissociation events can be monitored in real-time. The Octet is capable of multi-step kinetic determination and will support up to 3 hours unattended operation.

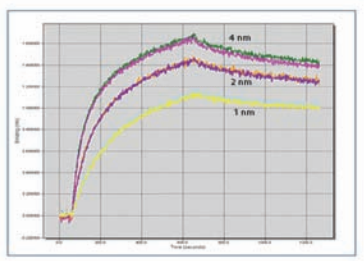

Figure 7. Human IgG binding to Protein A on an Octet Streptavidin Biosensor

Quantitation

With the Octet System, quantitation is determined by measuring the rate of increase in optical thickness as the sample binds to the sensor. Different protein concentrations result in different binding curves, as shown in Figure 8.

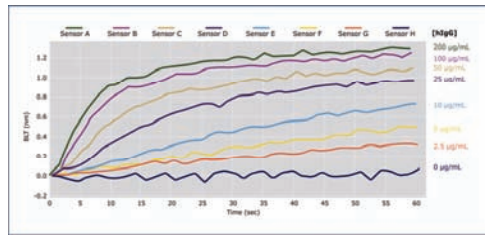

Figure 8. Raw binding charts from an Octet quantitation assay

The rate of binding to a capture molecule is presented in real-time and a standard curve is derived automatically from the different binding rates. This standard curve is used to calculate the concentration of unknown samples.

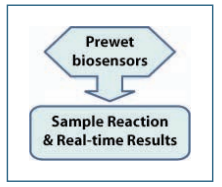

Figure 9. Octet Protocol

The Octet System is designed to streamline antibody development and production by providing results in real-time. For quantitation applications, a simple protocol with real-time processing provides results in minutes.

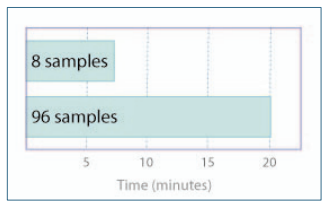

Figure 10. Octet throughput for quantitation applications

Test samples are assayed on the Octet System using SBS standard, black 96-well microplates. The Octet System is configured with an 8-channel biosensor manifold to process up to 8 samples in parallel and a maximum of 96 samples unattended. In quantitation mode this allows for the complete analysis of 96 samples in under 20 minutes *(Figure 10)*.

SUMMARY

To meet the resurgence of interest in antibody and protein therapeutics development, the Octet system from ForteBio provides a streamlined method for quantitation and kinetics measurement. Specifically, Octet provides:

- Label-free detection
- Real-time results
- Minimal interference from media
- Compatibility with crude lysates
- Automation

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