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Use of Dip and Read™ Anti-FLAG Biosensors for Rapid Quantitation and Kinetic Analysis of FLAG® Proteins

Weilei Ma*, Darick Dayne, Linda Jun, Ling Zhang, Bettina Heidecker, Tania Garcia, Tim Barnabei, Robert Wicke and Krista L. Witte
ForteBio, A Division of Pall Corporation, 1360 Willow Road, Suite 201, Menlo Park, CA 94025
www.fortebio.com

Abstract

Specific detection, quantitation and kinetic analysis of FLAG proteins can be performed using ForteBio's new Anti-FLAG biosensors. The biosensor can be used in crude samples, allowing for the specific capture of FLAG proteins without the need for purification. Data from both quantitation and kinetic applications is presented below.

Introduction

Anti-FLAG Biosensors. The Anti-FLAG Biosensor consists of Sigma's high-affinity ANTI-FLAG® M2 antibody pre-immobilized onto a ForteBio biosensor. The biosensor can capture and immobilize FLAG proteins for both quantitation and kinetics applications.

In quantitation applications, binding of the FLAG protein to the biosensor is monitored in real time using an Octet® or BLItz® system and can be compared to the binding of a known standard to determine concentration. With appropriate dilution, it is possible to measure analytes in complex matrix conditions.

In kinetics applications, the binding and dissociation of the target analyte to the immobilized FLAG proteins can be monitored in real time and can be used to determine the kinetic constants for association and dissociation. The surface is well suited for capture and analysis directly from complex mixtures and is a good alternative to chemical protocols such as EDC/NHS and biotinylation.

Bio-layer Interferometry (BLI) Technology. BLI is an analytical technique that monitors the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on the biosensor tip, and an internal reference layer (Figure 1). Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern. This change, measured in nanometers (nm), is reported in real time.

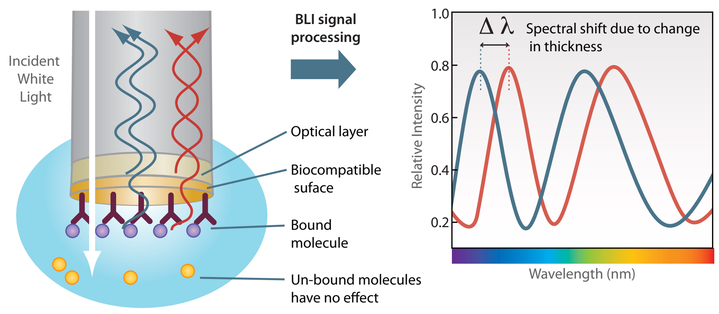


FIGURE 1: Bio-layer Interferometry (BLI) Technology Principle.

Quantitation Assay

The amount of FLAG protein can be quantitated in both pure and crude samples. The quantitation of FLAG protein is based on the binding kinetics of the FLAG protein to the Anti-FLAG Biosensor. Since each FLAG protein may differ in binding kinetics due to differences in sequence or sterics, it is important to use a standard as similar to the unknown as possible.

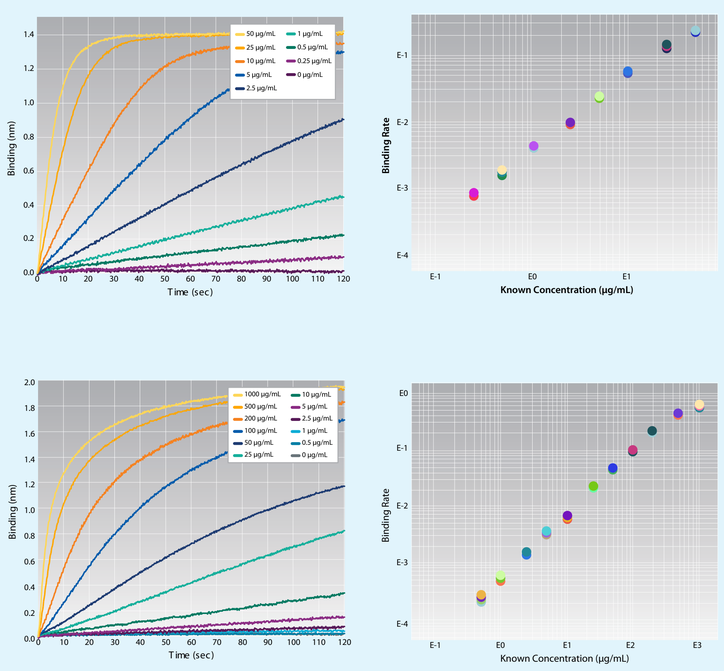


FIGURE 2: Analysis of TRAIL-FLAG and α1PDX-FLAG proteins using Anti-FLAG biosensors on the Octet RED384 instrument. Analysis was performed in Sample Diluent at concentrations specified in Table 1. Real-time binding curves (left) and resulting dose response curve (right) are shown.

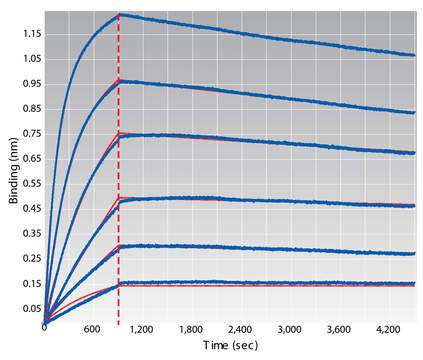
TABLE 1: Dynamic range and precision data from TRAIL-FLAG and α1PDX-FLAG proteins.

	Analyte Detected	TRAIL-FLAG (MW 23 kDa)		α1PDX-FLAG (MW 47 kDa)	
		BR (nm/s)	%CV	BR (nm/s)	%CV
	1000 mg/mL			0.6305	7.0%
	500 mg/mL			0.4249	3.0%
	200 mg/mL			0.2056	2.4%
	100 mg/mL			0.0982	2.6%
Dynamic Range	50 mg/mL	0.2340	3.0%	0.0459	6.4%
	25 mg/mL	0.1363	2.9%	0.0226	1.3%
	10 mg/mL	0.0580	2.6%	0.0065	5.0%
	5 mg/mL	0.0231	1.5%	0.0033	5.4%
	2.5 mg/mL	0.0093	2.1%	0.0014	5.6%
	1.0 mg/mL	0.0041	3.9%	0.0005	12.0%
	0.5 mg/mL	0.0019	7.2%	0.0002	14.3%
	0.25 µg/mL	0.0009	7.2%		
	0.00 mg/mL	0.0000	—	0.0000	—

Materials and Methods. Anti-FLAG biosensors (ForteBio, PN 18-5110) were hydrated in Sample Diluent (ForteBio, PN 18-1000) for a minimum of 10 minutes prior to use. The following two FLAG proteins were analyzed on Anti-FLAG biosensors: TRAIL-FLAG (AdipoGen, PN AG-40B-0003AA) and α1PDX-FLAG (Calbiochem, PN 126850). Typical analysis was performed by diluting analyte into Sample Diluent at specified concentrations as shown in Table I. Each sample was analyzed in triplicate with a read time of 120 seconds at 1000 rpm on the Octet RED384 instrument. Octet Data Analysis 7.0 software was used to calculate the binding rates (BR). Coefficients of variation (%CV) were calculated based on the triplicate assays. Dynamic range was defined as a %CV of less than 15% and a separation of at least three times standard deviation from neighboring concentrations.

Kinetics Assay

The ability to characterize the interaction between proteins can provide valuable information for further research decisions. Simply by inspecting real time binding curves, information on binding and dissociation can be determined. The association and dissociation curves of TRAIL-FLAG protein binding to an anti-TRAIL antibody analyzed on Anti-FLAG biosensors are shown in Figure 3.



k_{on}	k_{off}	K_D
3.08 E-10 M	1.04 E+ 05 1/Ms	3.21 E-05 1/s

Materials and Methods. Kinetics analysis of TRAIL-FLAG protein (AdipoGen, PN AG-40B-0003AA) binding to Mouse anti-TRAIL antibody (R&D Systems, PN MAB3751) using Anti-FLAG biosensors on Octet RED384 Instruments. Anti-FLAG biosensors were hydrated for 10 minutes in 10X Kinetics Buffer (Fortebio, PN 03-0040) prior to analysis. Assay steps include 3 minutes of equilibration, 10 minutes capture of FLAG protein, 5 minutes of baseline, 15 minutes of analyte association and 60 minutes of analyte dissociation. Analyte concentrations were 0, 1.25, 2.5, 5.0, 10, 20, and 40 nM. 10X Kinetics Buffer was used as the matrix throughout. Data were processed and curve fit using a 1:1 binding model with global fitting by Octet Data Analysis 7.0 software. The kinetics results are reported correspondingly in Figure 3.

FIGURE 3: Kinetic analysis of TRAIL-FLAG protein binding to an anti-TRAIL antibody on Anti-FLAG biosensors.

Minimum Recommended Sample Dilution for Quantitation Assays

Sample Type	Recommended Dilution
Purified proteins	Dilute into assay range
Samples from column eluents	Dilute into assay range
Serum-free cell culture supernatants media	Neat or two-fold (2X)
Serum containing cell culture supernatants media	Neat
Bacterial cell pellet lysed by sonication	Ten-fold (10X)
Bacterial cell pellet lysed by B-PER	Twenty-fold (20X)

Dilution Linearity and Spike Recovery of FLAG Protein

A dilution linearity and spike recovery study of the FLAG protein in sample diluent shows ±10% of expected concentration through the assay dynamic range, and reveals a good correlation curve between expected concentration and calculated concentration.

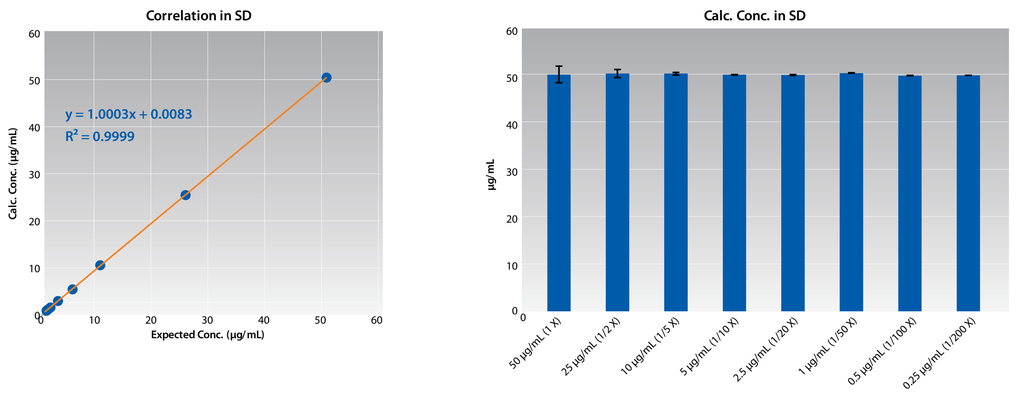


FIGURE 4: Dilution linearity and spike recovery of TRAIL-FLAG protein in Sample Diluent (ForteBio PN 18-1000) using Anti-FLAG biosensors on the Octet RED384 instrument.

Materials and Methods. TRAIL-FLAG protein was spiked in Sample Diluent (ForteBio PN 18-1000) at 50 µg/mL and serial dilutions were made for 25, 10, 5, 2.5, 1, 0.5, and 0.25 µg/mL concentrations. The biosensors were hydrated prior to assaying samples. Each sample was then analyzed in triplicate with a read time of 120 seconds at 1000 rpm on the Octet RED384 instrument.

Performance on Multiple Instrument Platforms

Anti-FLAG biosensors can be used on all ForteBio instrument platforms with comparable performance.

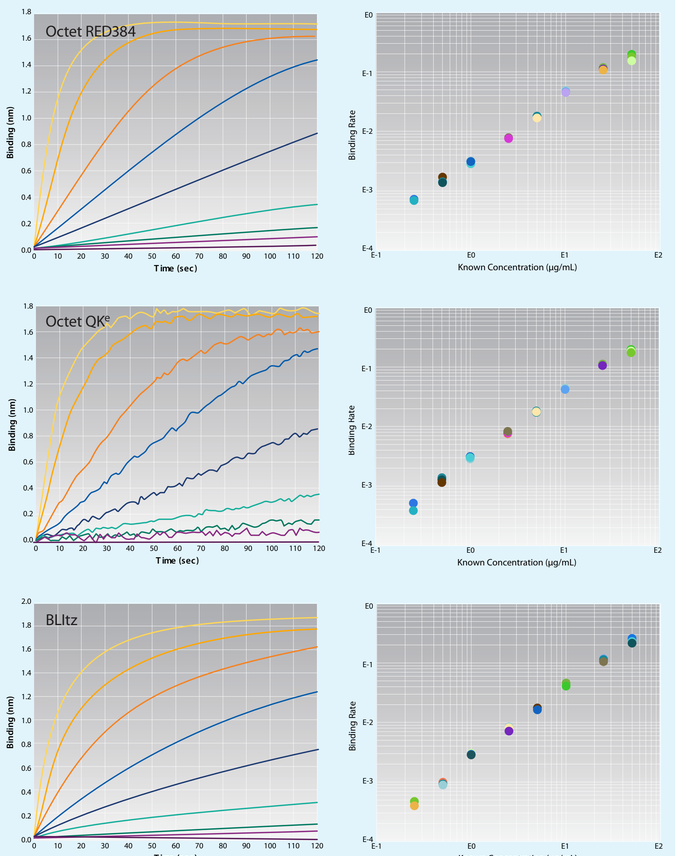


TABLE 2: Dynamic range and precision data from TRAIL-FLAG protein on multiple instruments.

	Instrument	Octet RED384		QK^e		BLItz	
		BR (nm/s)	%CV	BR (nm/s)	%CV	BR (nm/s)	%CV
	50 mg/mL	0.1894	7.5%	0.1873	6.7%	0.2364	8.5%
	25 mg/mL	0.1125	4.9%	0.1083	1.8%	0.1114	5.1%
	10 mg/mL	0.0470	2.9%	0.0428	3.7%	0.0427	7.1%
Dynamic Range	5.0 mg/mL	0.0175	4.7%	0.0176	3.0%	0.0172	4.4%
	2.5 mg/mL	0.0077	2.0%	0.0077	3.7%	0.0080	8.9%
	1.0 mg/mL	0.0031	5.6%	0.0029	2.4%	0.0030	3.2%
	0.5 mg/mL	0.0015	9.6%	0.0012	8.7%	0.0009	5.2%
	0.25 mg/mL	0.0007	2.9%	0.0005	14.4%	0.0004	12.0%
	0.00 mg/mL	0.0000	—	0.0000	—	0.0000	—

Materials and Methods. Anti-FLAG biosensors (ForteBio PN 18-5110) were hydrated in Sample Diluent (ForteBio PN 18-1000) for a minimum of 10 minutes prior to use. TRAIL-FLAG protein (AdipoGen, PN AG-40B-0003AA) was analyzed on Anti-FLAG biosensors on the Octet RED384, Octet QK^e and the BLItz systems. Analysis was performed by diluting analyte into Sample Diluent at specified concentrations as shown in Table 2. Each sample was analyzed in triplicate with a read time of 120 seconds at 1000 rpm. Octet Data Analysis 7.0 software was used to calculate the binding rates (BR). Coefficients of variation (%CV) were calculated based on the triplicate assays. Dynamic range was defined as a %CV of less than 15% and a separation of at least three times standard deviation from neighboring concentrations.

FIGURE 5: Triplicate analysis of TRAIL-FLAG protein using Anti-FLAG biosensors on the Octet RED384, Octet QK^e and the BLItz systems. Analysis was performed in Sample Diluent at concentrations specified in Table 2. Real-time binding curves (left) and resulting dose response curve (right) are shown.

Conclusions

- Anti-FLAG biosensors can be used for specific detection, purification, quantitation and kinetic characterization of FLAG proteins.
- Anti-FLAG biosensors enable quantitation and detection of FLAG proteins in purified or diluted crude samples (see Quantitation Assay section). The results shown demonstrate that quantitation using BLI technology, ForteBio's instrument platforms and Anti-FLAG biosensors provides a direct, specific and rapid means of quantifying FLAG proteins without the need for secondary amplification reagents. The protocol is very simple and involves no wash steps.
- Anti-FLAG biosensors also enable kinetic characterization of molecular interactions between FLAG proteins and their target analytes (see Kinetics Assay section).
- The Anti-FLAG Biosensor complements other pre-fabricated and custom biosensors on the Octet or BLItz platforms to provide a comprehensive set of tools for use across the entire bioprocess workflow, from cell line development to production monitoring and quality analysis.

*Author for correspondence: weilei_ma@pall.com