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Product Datasheet

Octet® ProL Biosensors

For Whole Molecule Antibody and Antibody Fragment Quantitation

Key Features

- Broader recognition of Ig isotypes and species than Protein A or G
- Regenerable and cost-effective format
- Binds whole molecule Ab and FAb fragments
- Quantitate in crude matrices

Overview

Dip and Read ProL (Protein L) Biosensors provide a rapid and direct method for quantifying a broad set of kappa light chain containing immunoglobulins, including whole molecules, FAb fragments and single chain variable fragments, in buffer, conditioned media or complex matrices. Protein L, which is factory-immobilized onto the biosensor, binds antibodies through the kappa light chain and recognizes a wider range of antibody classes than either Protein A or Protein G, including IgG, IgM, IgA, IgE and IgD. Due to its specificity, Protein L recognizes mouse VkI (but not VkII, VkIII and VkIV) and human VkI, VkIII and VkIV (but not VkII). The ProL Biosensor is especially useful for quantifying antibodies and antibody fragments from serum based cultures because Protein L does not bind bovine immunoglobulins, which often contaminate serum supplements.

Quick Facts

- Typical dynamic range for hFAb:
  - 0.05–2000 µg/mL (Octet® R8, RH16 and RH96 systems)
- Sensitivity can be extended by longer read times and higher shake speed.
- Throughput:
  - 16 and 96 samples in as little as 2 minutes on Octet® RH16 and Octet® RH96 respectively
  - Precision: < 10% CVs

Bioprocessing Applications Examples

- Quantitation of IgG's via kappa light chain
- Hybridoma screening
- Clone selection
- Antibody and Fab fragment titer
- Media development
- Bioreactor growth optimization
- Chromatography mass balance

Principle of Antibody Quantitation

Protein L is an immunoglobulin binding protein originally isolated from *Peptostreptoccus magnus* that binds the kappa light chains of most antibodies and antibody fragments. Immunoglobulin binding to the ProL Biosensor alters the properties of light on the biosensor surface, allowing the association event to be monitored in real time using the Octet® system. Higher antibody concentrations cause faster binding rates. Analyte concentration is extrapolated from a standard curve. Due to the high specificity of Protein L for the antibody light chain, ProL Biosensors can directly quantitate analytes in conditioned media and complex matrices.

Wide Dynamic Range

For most human antibodies and antibody fragments, Protein L detects analytes at concentrations from 50 ng/mL to 2 mg/mL with variation of the assay read time and shake speed (Figure 1). The ability to tune the assay over this wide dynamic range reduces the need for multiple dilutions, increasing the number of samples per plate and assay efficiencies.

Table 1: Proteins A, G and L possess unique affinity profiles toward antibodies from different species and isotypes. Protein L binds with high affinity to more species and isotypes than Proteins A and G, broadening its potential applications.

Antibody	Protein L	Protein A	Protein G
Rat Total IgG	s	w	m
Rat IgG1	s	w	m
Rat IgG2a	s	nb	s
Rat IgG2b	s	nb	w
Rat IgG2c	s	s	s
Rabbit IgG	w	s	s
Mouse IgG1	s	w	m
Mouse IgG2a	s	s	s
Mouse IgG2b	s	s	s
Mouse IgG3	s	s	s
Goat IgG	nb	w	s
Sheep IgG	nb	w	s
Bovine IgG	nb	w	s
Guinea Pig IgG	w	s	w
Hamster IgG	s	m	m
Pig IgG	s	s	w
Horse IgG	–	w	s
Donkey IgG	–	m	s
Dog IgG	–	s	w
Cat IgG	–	s	w
Human IgG1	s	s	s
Human IgG2	s	s	s
Human IgG3	s	w	s
Human IgG4	s	s	s

s = strong m = medium w = weak nb = no binding

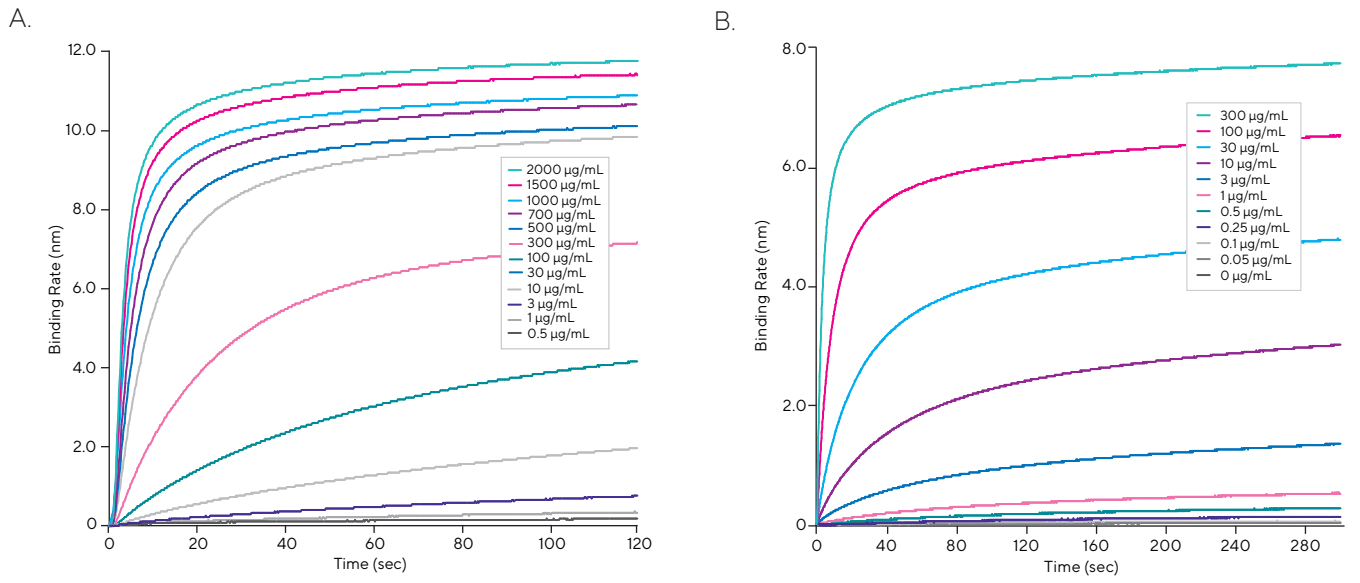

Figure 1: Octet® ProL detects a human IgG FAb over a wide dynamic range from 0.5–2000 µg/mL in sample diluent using parameters tuned for high (A) and low (B) concentrations. High concentration samples (0.5–2000 µg/mL, Panel A) were shaken at 400 rpm. Low concentration samples (0.05-300 µg/mL, Panel B) were shaken at 1000 rpm.

Table 2: Three Octet® ProL Biosensors were used to repeatedly analyze samples of 10 µg/mL human IgG FAb in neat CHO DG44 medium at a shake rate of 400 rpm. The Octet® ProL Biosensors were pre-conditioned with 3 regeneration cycles prior to the first measurement (R0) and regenerated in 10 mM Glycine pH 1.5 between each subsequent measurement (R0 through R9). After 10 regeneration cycles, the loss of capacity was less than 4% and the coefficient of variance was less than 2%, demonstrating efficient recovery of capacity and high reproducibility.

Biosensor #	Concentration (µg/mL) of hFAb detected per regeneration cycle										Statistics			
	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	Avg.	Std. Dev.	CV%	% Loss
1	10.2	10.1	10.0	10.0	10.0	10.0	9.91	9.87	9.92	9.85	9.99	0.11	1.1%	-3.4%
2	10.3	10.2	10.1	10.1	10.1	10.1	10.0	9.96	10.0	9.90	10.1	0.12	1.2%	-3.9%
3	10.4	10.3	10.3	10.3	10.2	10.2	10.2	10.1	10.1	10.1	10.2	0.10	1.0%	-2.9%

Cost-Effective Regeneration

Octet® ProL Biosensors can be regenerated up to ten times or more via a standard low-pH protocol. Regeneration dissociates the bound antibody from the ProL Biosensor, allowing additional analyses and providing a cost-effective format for analyzing large sample libraries. Table 2 summarizes the high recovery and precision of three representative biosensors over ten regeneration cycles.

Ordering Information

Part No.	UOM	Description
18-5085	Tray	96 Octet® ProL Biosensors coated with Protein L (recommended Sample Diluent sold separately)
18-5086	Pack	Five trays of 96 Octet® ProL Biosensors coated with Protein L (recommended Sample Diluent sold separately)
18-5087	Case	Twenty trays of 96 Octet® ProL Biosensors coated with Protein L (recommended Sample Diluent sold separately)

Note: A purified standard that is identical to the experimental samples is required.

ProA (Protein A) and ProG (Protein G) Biosensors are also available.

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