SARTORIUS

Product Datasheet

Octet® SSA Biosensors

For Analysis of Small Molecule-Protein Interactions

Key Features

- Enables immobilization of any protein in high density via streptavidin
- Designed for use in small-molecule and fragment screening and kinetic characterization

Quick Facts

- Immobilization Chemistry: High-density streptavidin
- Baseline Stability: 60 minutes
- Number of Acceptable Regeneration Cycles: protein dependent

Overview

Small molecule kinetics can be rapidly measured in high throughput on the Octet® instruments. In a typical experiment, a biotinylated protein target is immobilized onto the Octet® high-capacity Super Streptavidin (SSA) Biosensor surface, and this surface is exposed to a solution of the small molecule in a microplate well. The association of the small molecule to the target protein on the biosensor is measured over time. Finally, the biosensor is moved to a well containing buffer to monitor the dissociation of the small molecule from the target protein. Rate constants can be calculated from the binding data, including on-rate (k_{on} or k_a), off-rate (k_{off} or k_d), and equilibrium dissociation constant (K_D).

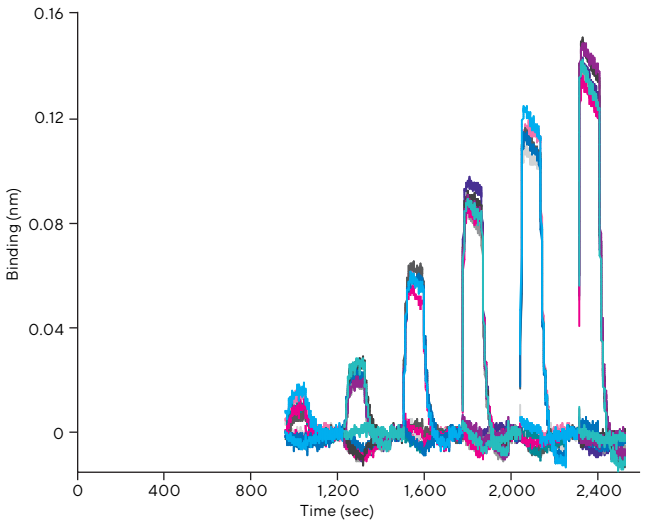

Figure 1: Processed data for the binding of furosemide (330 Da) to carbonic anhydrase. Data shown is a result of the subtraction of the biocytin reference biosensor data from the carbonic anhydrase biosensor data. The binding of furosemide at 0.12, 0.37, 1.1, 3, 10, and 30 µM is clearly visible.

Use of the Octet® SSA Biosensors

When performing small molecule analysis on the Octet® system, each Octet® SSA Biosensor can be used for multiple analyses since most small molecules have affinities greater than 1 nM and thus dissociate fully after several minutes. Typical experiments include both biosensors immobilized with the biotinylated target protein (target biosensors) and biosensors blocked with biocytin (reference biosensors). Figure 1 shows data for the binding of furosemide (330 Daltons) to carbonic anhydrase collected on an Octet® R8 system. The precision for a typical run on the Octet® R8 system is shown in Table 1.

Data Analysis

Analysis of small molecule kinetic data is easy using the Octet® BLI Analysis Software. The software supports both Global analysis and Steady State analysis of kinetic data sets. Global analysis derives a single set of parameters including R_{max}, k_{on}, k_{off}, and K_D from a set of association and dissociation curves from a concentration series (see the *Octet® Data Analysis User Guide* for more information). This method generates more precise and accurate data than results obtained from association and dissociation data from a single concentration.

An example of global fitting of a titration series is shown in Figure 2. The K_D can also be derived from equilibrium responses using Steady State analysis. This method does not generate k_{on} and k_{off} values. The experiment requires a concentration-dependent response, and dilutions of 2–4X are recommended for a six-point concentration series (minimum). An example is shown in Figure 2 (right).

Table 1: Precision of furosemide analysis with carbonic anhydrase on the Octet® R8 system.

Analysis	R_{max} (Δnm)	R_{max} Error	k_{off}(1/s)	k_{on}(1/Ms)	k_{on} Error	K_D (M)	Chi²	R²
1	0.0975	0.0001	7.83E-02	6.49E+04	4.57E+02	1.21E-06	0.053	0.99
2	0.1017	0.0001	7.88E-02	5.75E+04	3.84E+02	1.37E-06	0.050	0.99
3	0.0951	0.0002	8.52E-02	6.76E+04	5.66E+02	1.26E-06	0.067	0.99
4	0.0976	0.0002	7.97E-02	6.20E+04	4.69E+02	1.28E-06	0.059	0.99
5	0.0931	0.0001	8.32E-02	8.72E+04	6.97E+02	9.54E-07	0.063	0.99
Avg	0.097	0.0002	0.081	67836	515	1.22E-06	0.058	0.989
SD	0.003	0.0002	0.003	11453	121	1.57E-07	0.007	0.002

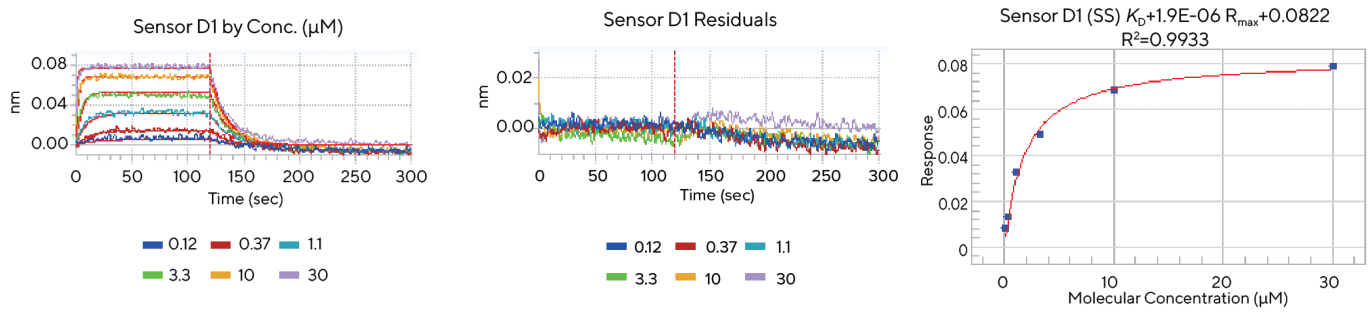

Figure 2: Global analysis of small molecule binding data at 0.12–30 µM (left), residuals for curve fitting (center), and the steady-state plot (right).

The Octet® SSA Biosensors are designed for use with the most senstive Octet® instruments, the Octet® R8, RH16 and RH96 systems. The latest version of software includes a predefined protocol to make this assay easy to run and is available with 21 CFR Part 11 compliance tools.

Ordering Information

Part No.	UOM	Description
18-5057	Tray	One tray of 96 Octet® SSA Biosensors coated with streptavidin for small molecule kinetic analysis
18-5065	Pack	Five trays of Octet® SSA Biosensors coated with streptavidin for small molecule kinetic analysis
18-5070	Case	Twenty trays of 96 Octet® SSA Biosensors coated with streptavidin for small molecule kinetic analysis

Note: Additional materials are required to run these assays. Please consult Technical Note 16, Small Molecule Binding Kinetics for full details.

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