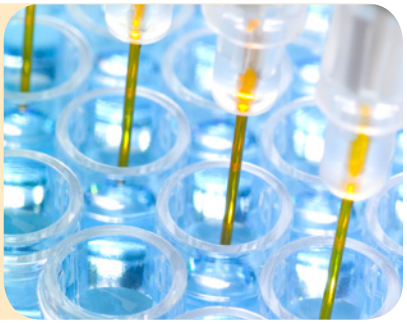
Amine Reactive (AR) Biosensors

Octet Biosensors for Label-Free Kinetic Analysis of Protein-Protein Interaction

Key Features

- Simple coupling of protein to biosensor via amide bond formation chemistry
- Biosensor can be regenerated and reused
- Designed for kinetic analysis of proteins with a MW > 30 kDa



ForteBio Amine Reactive (AR) biosensors are a flexible tool for large molecule kinetic analysis and kinetic screening for large proteins of interest with a primary amine. These AR biosensors enable the coupling of proteins to carboxylate groups on the biosensor surface via accessible amine groups within the target protein. The coupling procedure is a simple three step protocol based on well characterized amide bond formation chemistry. Amine coupling can be fully automated online or can be batch immobilized offline. Once the protein is immobilized onto the biosensor, it can be used on the Octet system to monitor in real-time, the binding of the target protein on the biosensor to its analyte in solution. Finally, regeneration conditions can be developed to enable reuse of the target protein on the biosensor for additional kinetic analyses.

QUICK FACTS

- **Method of Immobilization:** Coupling free amine groups on the target protein to carboxylate groups on the biosensor surface
- **Baseline Stability:** 60 minutes
- **Number of Regeneration Cycles:** protein dependent

USE OF AMINE REACTIVE BIOSENSORS

The Amine Reactive biosensor surface is a biocompatible layer with many available carboxylic acid groups. Treatment of this surface with an EDC/NHS mixture activates the surface toward nucleophillic attack. Subsequent exposure of a protein at a pH below its pI will result in an amide bond formation between the primary amine of the protein and the carboxylate of the biosensor surface. Once the protein is covalently bound to the biosensor it can be further analyzed for interactions with other proteins for kinetic analysis (Figure 1). Proteins can also be immobilized to these biosensors in batch. Please consult Technical Note #7, *Batch Immobilization of Protein Onto Amine Reactive Biosensors*. Note that due to the density of carboxylic acid groups, this biosensor is best suited for kinetic analysis of proteins with a molecular weight of 30 kDa or larger.

REGENERATION AND REUSE OF AR BIOSENSORS

In some applications, particularly kinetic screening, it may be advantageous to assay several analyte samples using the same ligand-coated biosensor. To accomplish this, the analyte must

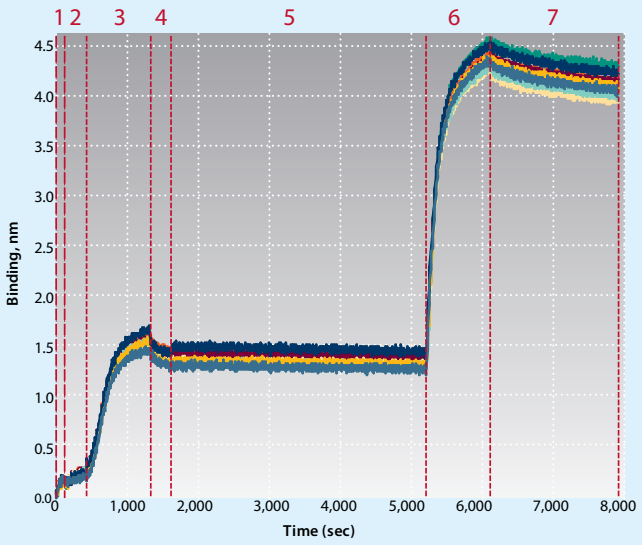


FIGURE 1: Immobilization of Protein A and binding of IgG to eight Amine Reactive biosensors in parallel on the Octet RED. The real time binding data shows all seven events during the experiment. 1 = baseline, 2 = activation with EDC/NHS, 3 = immobilization of Protein A, 4 = quench with 1M ethanolamine, 5 = baseline, 6 = association of human IgG, 7 = dissociation of human IgG.

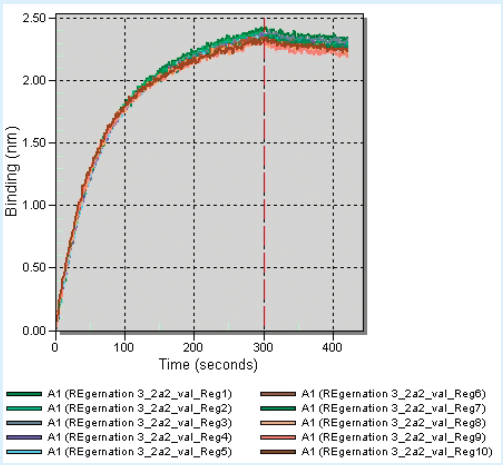


FIGURE 2A: Real-time binding chart of the development of regeneration conditions for Protein A-coated Amine Reactive biosensors binding to human IgG. Note no significant loss in binding capacity over 9 regenerations.

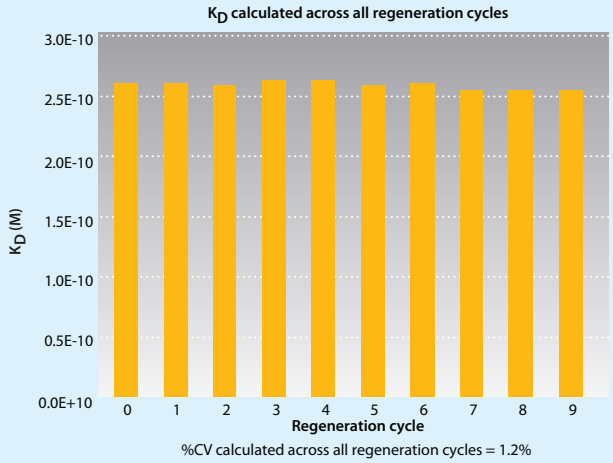


FIGURE 2B: K_D values calculated for each set of binding curves in Figure 2A using the Octet software. The reported CV is the coefficient of variance of the K_D from each set of binding curves across all regeneration cycles.

be dissociated from the ligand-coated biosensor, regenerating the biosensor so that it can be used in another assay. There are different modes of interactions between ligand-analyte pairs (for example, hydrophobic forces, ionic binding). As a result, the conditions that disrupt these interactions are protein dependent and the regeneration protocol for a particular ligand-analyte pair must be determined empirically. For details on how to determine the best regeneration conditions, please consult Technical Note #8, *Regeneration Strategies for Amine Reactive Biosensors on the Octet System*. An example of data generated before and after biosensor regeneration is shown in Figure 2.

TYPICAL ASSAY PARAMETERS

- **Sample Volume:** 200 µL/well for 96-well plate and 80 µL/well for 384-well plate (post-dilution)
- **Hydration Sample Volume:** 200 µL/well (post-dilution)
- **Flow Rate:** 1000 rpm
- **Biosensor Hydration and Sample Plate Equilibration:** 10 minutes
- **Companion Product:** Amine Coupling Reagent Kit (ForteBio part no. 18-5017)

Dip and Read Amine Reactive biosensors are compatible with all Octet™ instruments including the new Octet 384 series. The latest version of software includes a predefined protocol to make this assay quick and easy to run and is available with 21 CFR Part 11 compliance tools.

ORDERING INFORMATION

Part No.	UOM	Description
18-5029	Tray	One tray of 96 Amine Reactive biosensors compatible with amine reactive coupling chemistry for kinetics analysis. Reagents sold separately.
18-5030	Pack	Five trays of 96 Amine Reactive biosensors compatible with amine reactive coupling chemistry for kinetics analysis. Reagents sold separately.
18-5031	Case	Twenty trays of 96 Amine Reactive biosensors compatible with amine reactive coupling chemistry for kinetics analysis. Reagents sold separately.
Note: additional materials are required to run these assays. Please consult Technical Notes 7 and 8 for full details.		

For more information about ForteBio's Octet platform for label-free, real-time detection of biomolecular interactions, applications, and services, visit www.fortebio.com or contact us directly.

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