Regeneration strategies for Streptavidin biosensors on the Octet platform

Developing a successful regeneration protocol

Overview

The ForteBio Octet® platform offers powerful tools for the real-time, label-free analysis of protein-protein interactions. Together, the Octet systems and Streptavidin (SA) biosensors provide a flexible format for kinetic analysis and kinetic screening for any biological interaction in which one molecule can be biotinylated.

In some applications, particularly kinetic screening, it may be advantageous to assay several protein samples using the same ligand-coated biosensor. To accomplish this, the target protein must be dissociated from the ligand-coated biosensor, regenerating the biosensor so that it can be used in another assay (Figure 1). This technical note provides guidelines to develop a successful regeneration protocol.

There are different modes of interaction between ligand and target protein pairs (*i.e.*, hydrophobic forces, ionic binding). As a result, the conditions that disrupt these interactions are protein dependent and the regeneration protocol for a particular ligand- target protein pair must be determined empirically.

To successfully regenerate the ligand-coated Streptavidin biosensor surface:

- Biosensor surface chemistry must be stable under the regeneration conditions.
- Immobilized ligand must be stable under the regeneration conditions and retain activity over multiple regeneration cycles (ligand-dependent).
- Ligand-target protein interaction must dissociate during regeneration (protein pair dependent).

The Octet system's standard microplate format provides a flexible platform for assays that incorporate regeneration. As shown in Figure 2, when the ligand is immobilized off-line and the assay includes regeneration, eight Streptavidin biosensors can be regenerated seven times for a total of sixty-four kinetic analyses.

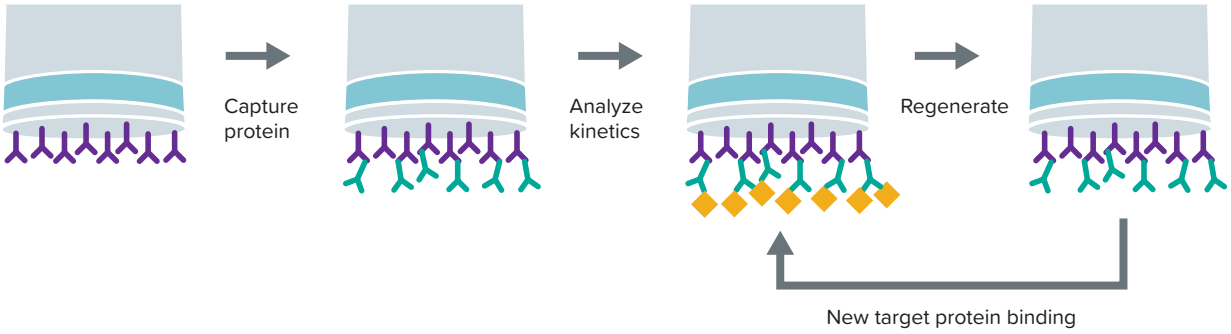

Figure 1: Regeneration of the Streptavidin biosensor disrupts the interaction between the immobilized ligand and the target protein to allow for subsequent binding cycles of new target proteins to the same surface.

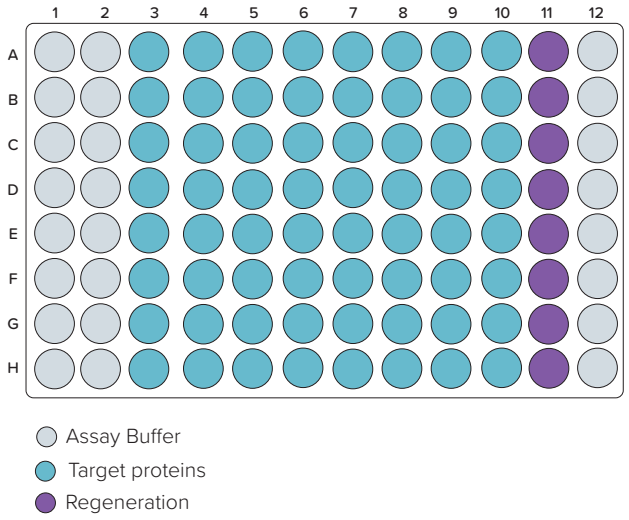

Figure 2: Example plate layout to allow analysis of up to 64 interactions using regeneration of 8 Streptavidin biosensors.

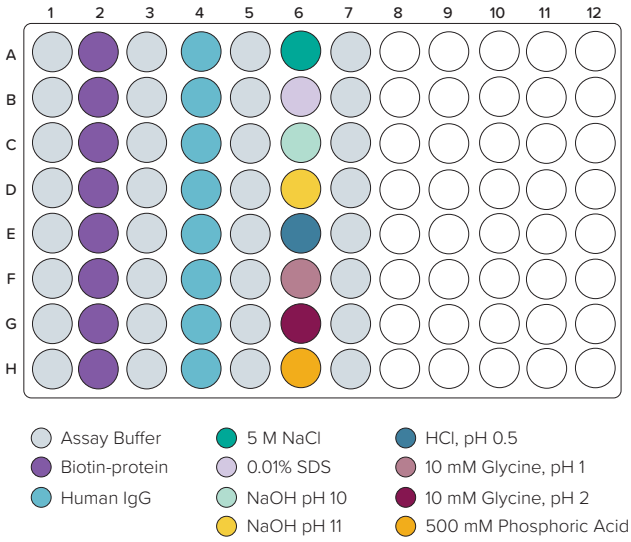

Figure 3: Example plate layout for screening and validatingup to 8 different regeneration reagents.

STABILITY OF THE STREPTAVIDIN BIOSENSOR SURFACE CHEMISTRY

The underlying surface chemistry of the Streptavidin biosensor is robust and stable over a broad range of pH and ionic strengths. This wide window of chemical tolerance enables many possible regeneration solutions. Most antibody-protein interactions can be disrupted using low pH buffers (pH 1–4), but some may require high pH or salt. Several commonly used regeneration conditions were tested as well as those frequently used on other biosensor platforms (Table 1).

Reagent	Maximum validated exposure time*
HCl (pH 0.5, 1.0, 1.5)	15 minutes
NaOH (pH 10, 11)	15 minutes
NaOH (pH 12, 12.5, 13)	Not recommended
10 mM Glycine (pH 1, 2, 3)	15 minutes
NaCl (1, 2.5, 5 M)	15 minutes
$MgCl_2$ (0.1, 0.5, 1 M)	15 minutes
Tween-20 (0.1%, 0.25%, 0.5%)	15 minutes
SDS (0.05%, 0.1%, 0.25%, 0.5%)	Not recommended
SDS (0.005%, 0.01%)	15 minutes
Phosphoric Acid (50, 100, 250, 500 mM)	15 minutes
EDTA (25, 50, 100 mM)	15 minutes
Triton X-100 (0.1%, 0.25%, 0.5%)	15 minutes

**Exposure times are for the Streptavidin biosensor itself. Stability of the protein immobilized will be protein-dependent.*

Table 1: Regeneration reagents tested with Streptavidin biosensor surface chemistry.

STRATEGY FOR DETERMINING REGENERATION CONDITIONS

Due to differences in protein structure and stability, the ability of the immobilized ligand to withstand a particular regeneration condition must be determined experimentally. By taking advantage of the flexibility of the Octet format, an assay can be designed to test and validate up to eight regeneration conditions in one run.

The general steps for developing and validating a regeneration method are given below.

1. Immobilize biotin-ligand on the Streptavidin Biosensors.
2. Bind the target protein to the ligand-coated biosensor.
3. Regenerate the biosensor using up to eight different regeneration reagents.
4. Bind the target protein at the same concentrations in Step 2.
5. Repeat the regeneration and rebinding steps for at least as many cycles as you expect to run in the final assay (for example, kinetics screening).
6. Identify the conditions that result in equivalent binding before and after regeneration throughout the desired number of binding/regeneration cycles.

Step	Reagent	Time (seconds)	Flow (rpm)	Step type
1*	Assay buffer	30–300	1000	Baseline
2	Biotin- ligand for immobilization	300–900	1000	Loading
3	Assay buffer	30–300	1000	Baseline
4	Target protein	300–900	1000	Association
5	Assay buffer	30–300	1000	Dissociation
6	Regeneration panel	5–30	1000	Regeneration
7	Running buffer	5–30	1000	Baseline
8	Regeneration panel	5–30	1000	Regeneration
9	Running buffer	5–30	1000	Baseline
10	Regeneration panel	5–30	1000	Regeneration
11	Running buffer	5–30	1000	Baseline
...	Repeat steps 3–11 for the desired number of binding/regeneration cycles to be validated			

** If the biotin-ligand is immobilized off-line, omit steps 1–2.*

Table 2: Regeneration screening and validation assay method.

Developing a regeneration method

REQUIRED MATERIALS

- Streptavidin Biosensors (ForteBio part nos. 18-5019, 18-5020 and 18-5021)
- Biotinylated ligand to be immobilized on the biosensor (biotinylation should be performed according to ForteBio Technical note TN-3006 or TN-3012)
- Target protein
- Assay buffer (PBS, HBS, etc. Should be kept consistent throughout the assay and regenerations)
- 96-well, flat bottom, black polypropylene microplates (Greiner Bio-One part no. 655209)

GENERAL CONSIDERATIONS

For each assay, identify seven or eight candidate regeneration conditions. For most antibody-protein interactions, low pH (pH 1–4) effectively regenerates the interaction. For other types of protein-protein interactions, high salt, high pH or detergents may be needed. In general, several short (*e.g.* 3–5 X 10 second) exposures to the regeneration buffer are more successful than a single, longer exposure.

There are many possible ways to configure a screening assay. For best efficiency, it is recommended that you immobilize the ligand onto eight Streptavidin biosensors and screen eight regeneration solutions in parallel to take advantage of the Octet System's throughput capacity.

SETTING UP THE ASSAY

1. Prepare the eight regeneration solutions to be tested.
2. Prepare 2 mL of target protein in the assay buffer at a concentration 10–20X greater than the expected Kd.
3. Transfer 200 µL of each reagent to the sample plate as shown in Figure 3.
4. Hydrate eight Streptavidin biosensors in the assay buffer following the instructions in the biosensor package insert.
5. Place the biosensors and the assay plate into the Octet instrument.
6. On the Octet instrument, program the assay method shown in Table 2.
7. Set the delay to 300 seconds and choose the "Shake while waiting" option.
8. Start the assay.
9. Once the assay is complete, use the data analysis program to overlay the original binding curve and the binding curves from the subsequent rounds of regeneration. If the regeneration condition is successful, the binding curves of each cycle will overlay with minimal change in the profile and will not show a loss in binding capacity when compared to earlier binding cycles (for example data, see Figure 6).

Example: determining and validating regeneration conditions for a receptor/protein interaction on Streptavidin biosensors

In this example, the optimal regeneration conditions for immobilized biotin-Protein A were determined. The objective was to identify the conditions that enable the regeneration of Protein A binding after hIgG association for at least eleven binding/regeneration cycles.

REQUIRED REAGENTS

- Assay Buffer (0.1 mg/mL BSA, 0.002% Tween-20, PBS)
- 2 mL of 5 µg/mL stock of biotin-Protein A in Assay Buffer
- 2 mL of 66 nM solution of hIgG in Assay Buffer
- 8 Streptavidin biosensors hydrated in Assay Buffer

REGENERATION CONDITIONS TESTED

- 5 M NaCl
- 0.01% SDS
- NaOH, pH 10
- NaOH, pH 11
- 500 mM phosphoric acid
- HCl, pH 0.5
- 10 mM glycine, pH 1
- 10 mM glycine, pH 2

ASSAY SETUP

The sample plate was set up as shown in Figure 4 and run on the Octet System using the assay method shown in Table 3.

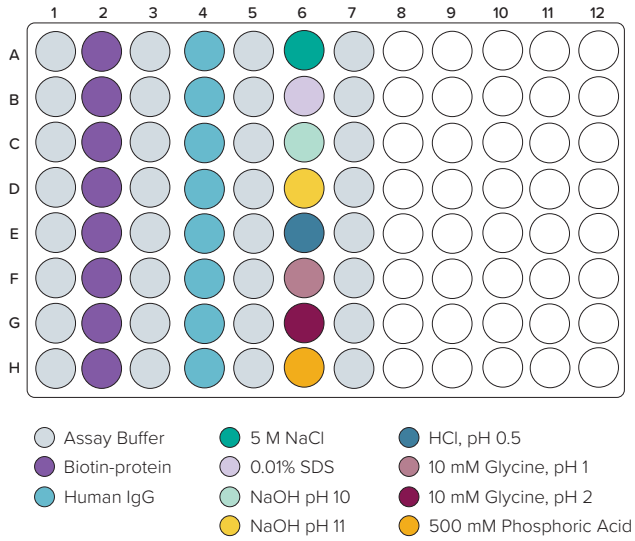

Figure 4: Plate layout for screening and validating 8 different regeneration reagents.

Step	Reagent	Time (sec)	Flow (rpm)	Step type
1	Assay Buffer	120	1000	Baseline
2	5 µg/mL biotin Protein A	120	1000	Loading
3	Assay Buffer	60	1000	Baseline
4	hIgG, 66 nM	120	1000	Association
5	Assay Buffer	120	1000	Dissociation
6	Regeneration panel	10	1000	Regeneration
7	Assay Buffer	10	1000	Baseline
8	Regeneration panel	10	1000	Regeneration
9	Assay Buffer	10	1000	Baseline
10	Regeneration panel	10	1000	Regeneration
11	Assay Buffer	60	1000	Baseline
...	Repeat steps 3–11 10 additional times			

Table 3: Screening assay method for Protein A regeneration conditions.

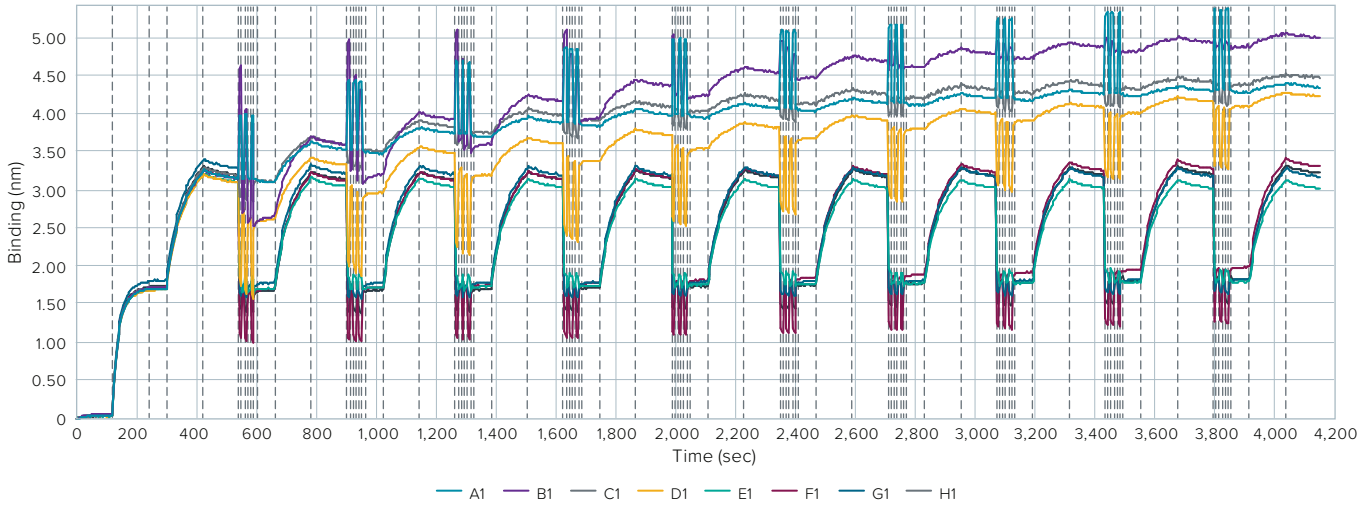

Figure 5: Real time results from regeneration scouting and validation experiment run on the Octet RED instrument. Data shown is from 8 Streptavidin biosensors with biotin-Protein A immobilized, binding human IgG and taken through regeneration with 8 different reagents over 11 binding cycles..

ASSAY RESULTS

In Figure 5, the real time binding chart for the assay shows significant differences in the effectiveness of the eight regeneration solutions across the eleven binding cycles tested. Only the acidic conditions (biosensors E1–H1) appear to show reasonable regeneration of the binding capacity after the first binding cycle.

Using the Data Analysis software, the successive binding cycles from each biosensor can easily be overlaid (Figure 6A–H). The best regeneration conditions are quickly assessed by determining the level of hIgG binding for biosensors across all binding cycles. In this example, the NaCl, SDS and basic regeneration solutions all show poor regeneration as is evident by the clear loss of the amount of hIgG binding from binding cycle 1 to 11 (Figure 6A–D). The conditions using HCl, glycine pH 1, glycine pH 2 and phosphoric acid all show good reproducibility of the hIgG binding across all eleven cycles (Figures 6E–H). In particular, the 500 mM phosphoric acid (biosensor H1) reagent shows very good regeneration of the protein A surface with a high level of reproducibility of hIgG binding throughout the assay.

From this single experiment a regeneration condition of 3X10 second cycles of 500 mM phosphoric acid has been identified as an excellent method for regenerating this receptor on Streptavidin biosensors.

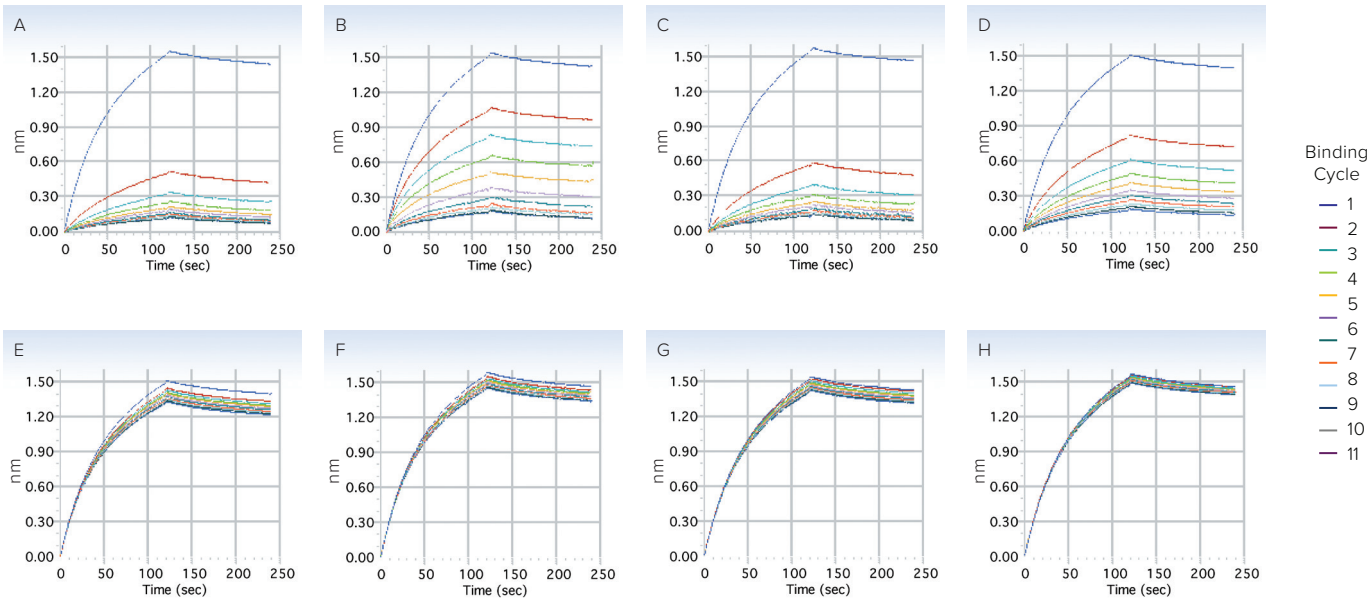

Figure 6: Analysis of effectiveness of eight different reagents in regenerating Protein A immobilized to Streptavidin biosensors. Reagents tested were: A) 5 M NaCl; B) 0.01% SDS; C) NaOH, pH 10; D) NaOH, pH 11; E) HCl pH 0.5; F) 10 mM Glycine pH 1; G) 10 mM glycine, pH 2; H) 500 mM phosphoric acid

ForteBio biosensor selection guide

	Anti-Human IgG Fc	Anti-Murine IgG Fc	Protein A	SA	SSA	AR	APS	AHC
Application: Quantitation (Q), Screening (S), Kinetics (K)	Q	Q	Q	Q, S, K	Q, S, K	S, K	K	K
Surface can be regenerated*	NO	NO	YES	YES	YES	YES	YES	YES
For small molecules (150–900 Da) on Octet RED	—	—	—	—	●	—	○	—
For peptides (500–2000 Da) on Octet RED	—	—	—	○	●	—	○	○
For small proteins (5–20 kDa)	—	◖	●	●	●	○	○	◖
For mid-sized proteins (20–150 kDa)	◖	●	●	●	●	◖	●	●
For large proteins (>150 kDa)	●	●	●	●	●	◖	●	●
Short kinetics assays (off rate <15 min)	—	—	—	●	●	◖	●	●
Long kinetics assays (off rate >15 min)	—	—	—	●	●	◖	◖	●
Custom quantitation assays	—	—	—	●	○	—	—	—
LOD <50 ng/mL, requires fast flow-rate and longer assay time	◖	◖	◖	◖	—	—	—	—
Upper limit of quantitation >1 mg/mL	—	—	◖	◖	—	—	—	—
Quantitation in serum-free crude cell lysates	●	●	●	●	—	—	—	—
Quantitation in serum-containing crude cell lysates	●	●	○	●	—	—	—	—
Quantitation in column elutes, buffer	●	●	●	●	—	—	—	—
High detergent concentrations (>1%)	●	●	●	●	●	○	●	○

— = Not Advised ○ = Good ◖ = Very Good ● = Excellent

*Dependent on chemistry of protein attached to the biosensor

Is this content is likely outdated? Do you want to keep this table as is, modify it for new biosensors or just nix the table completely and refer folks to the most current biosensor selection guides online?

FORTÉBIO

www.fortebio.com

ForteBio
47661 Fremont Boulevard
Fremont, CA 94538
888.OCTET-75 or 650.322.1360
fortebio.info@moldev.com

ForteBio Analytics (Shanghai) Co., Ltd.
No. 88 Shang Ke Road
Zhangjiang Hi-tech Park
Shanghai, China 201210
salesops.china@moldev.com

Molecular Devices (UK) Ltd.
660-665 Eskdale
Winnersh Triangle
Wokingham, Berkshire
RG41 5TS, United Kingdom
+44 118 944 8000
uk@moldev.com

Molecular Devices (Germany) GmbH
Bismarckring 39
88400 Biberach an der Riss
Germany
+ 00800 665 32860

P/N TN-3014 Rev B