

## Regeneration Strategies for Streptavidin Biosensors on the Octet® Platform

Developing a successful regeneration protocol



### Technical Note

#### Scope

This Technical Note on Octet® Streptavidin (SA) Biosensors provides insight into the strategy for determining and validating regeneration conditions and optimizing the number of regeneration cycles on the biosensor.

#### Keywords or phrases:

Octet®, Bio-Layer Interferometry, BLI, biotin-protein interaction, biosensor regeneration, optimization, Streptavidin

### Executive Summary

The 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 the ligand 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

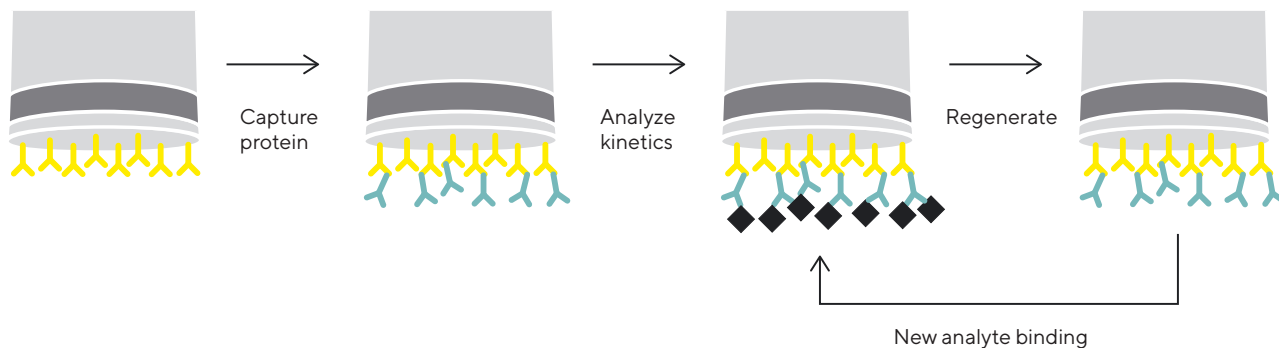
biosensor, regenerating the biosensor so that it can be used in further assays (Figure 1). This technical note provides guidelines to develop a successful regeneration protocol.

There are different modes of interaction between the ligand and target analyte pairs (e.g., 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.

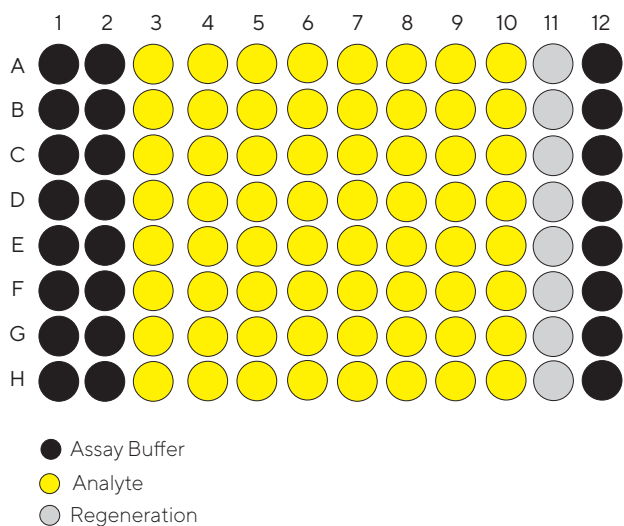
To successfully regenerate the ligand-coated Streptavidin Biosensor surface:

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

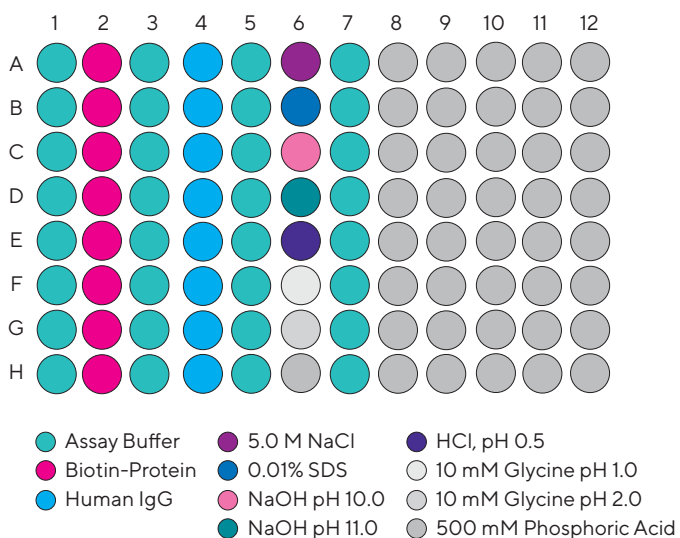
The Octet® system's standard microplate format provides a flexible platform for assays that incorporate regeneration. As shown in Figure 2, when the ligand is captured off-line and the assay includes regeneration, eight Streptavidin Biosensors can be regenerated seven times for a total of 64 kinetic analyses.



**Figure 1:** Regeneration of the Streptavidin Biosensor disrupts the interaction between the captured ligand and the analyte protein to allow for subsequent binding cycles of new analyte 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 validating up 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 the use of many possible regeneration solutions. Most antibody-protein interactions can be disrupted using low pH buffers (pH 1–4), but some may require higher pH or salt solutions. Several commonly used regeneration conditions were tested as well as those frequently used on other biosensor platforms (Table 1).

**Table 1:** Regeneration reagents tested with Streptavidin Biosensor surface chemistry.

| Reagent                                | Maximum validated exposure time* |
|--|----------------------------------|
| HCl (pH 0.5, 1.0, 1.5)                 | 15 minutes                       |
| NaOH (pH 10.0, 11.0)                   | 15 minutes                       |
| NaOH (pH 12.0, 12.5, 13.0)             | Not recommended                  |
| 10 mM Glycine (pH 1.0, 2.0, 3.0)       | 15 minutes                       |
| NaCl (1.0, 2.5, 5.0 M)                 | 15 minutes                       |
| MgCl <sub>2</sub> (0.1, 0.5, 1.0 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 captured will be protein-dependent.

## Strategy for Determining Regeneration Conditions

Due to differences in protein structure and stability, the ability of the captured 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 qualifying a regeneration method are given below.

1. Capture biotin-ligand on the Streptavidin Biosensors.
2. Bind the analyte to the ligand-coated biosensor.
3. Regenerate the biosensor using up to eight different regeneration reagents.
4. Bind the analyte 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.

Table 2: Regeneration screening and validation assay method.

| Step | Reagent   | Time (seconds) | Shaker speed (rpm) | Step type    |
|------|---|----------------|--------------------|--------------|
| 1*   | Assay buffer  | 30-300         | 1000               | Baseline     |
| 2    | Biotin- ligand for capture  | 300-900        | 1000               | Loading      |
| 3    | Assay buffer  | 30-300         | 1000               | Baseline     |
| 4    | Analyte   | 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.

## Developing a Regeneration Method Required Materials

- Streptavidin Biosensors (part nos. 18-5019, 18-5020 and 18-5021)
- Biotinylated ligand to be captured on the biosensor (biotinylation should be performed according to Technical Note TN-3006 or TN-3012)
- Analyte
- 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 1.0 – 4.0) 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 capture 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 analyte 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 captured biotin-Protein A were determined. The objective was to identify the conditions that enable the regeneration of Protein A binding after human IgG (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

- 5.0 M NaCl
- 0.01% SDS
- NaOH, pH 10.0
- NaOH, pH 11.0
- 500 mM phosphoric acid
- HCl, pH 0.5
- 10 mM glycine, pH 1.0
- 10 mM glycine, pH 2.0

## 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.

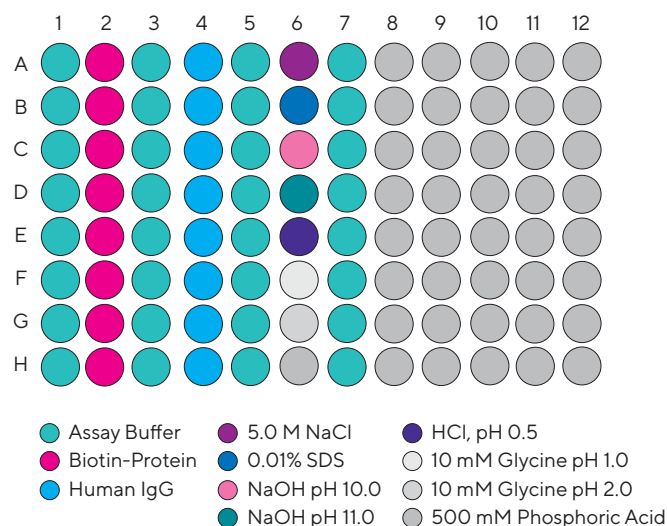


Figure 4: Plate layout for screening and validating 8 different regeneration reagents.

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

| Step | Reagent                               | Time (sec) | Shaker speed (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 |            |                    |              |

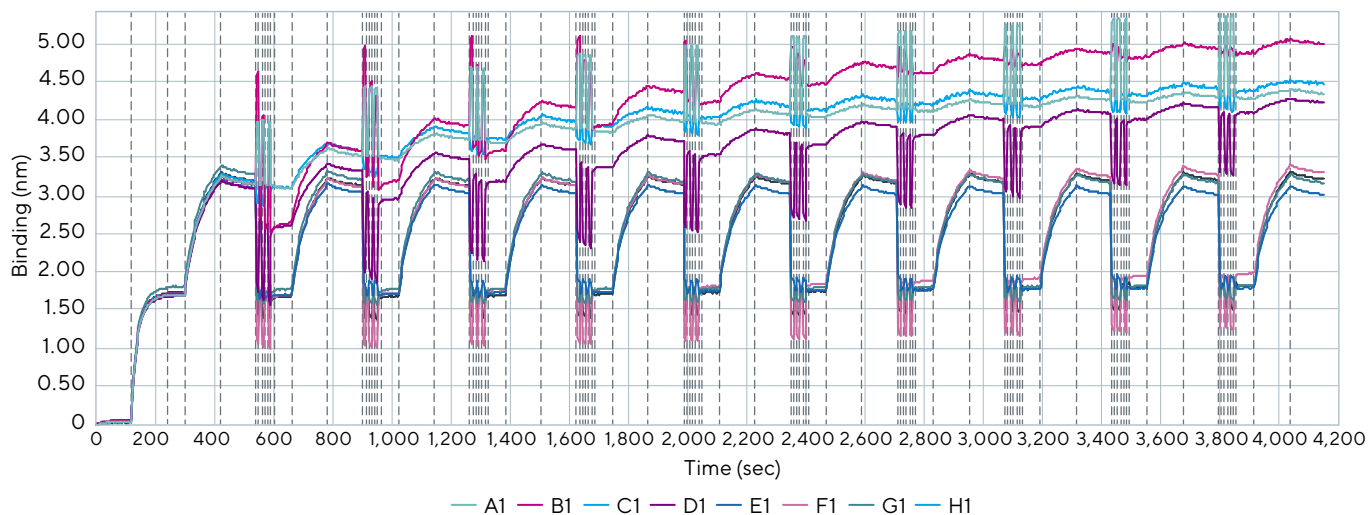


Figure 5: Real time results from regeneration scouting and validation experiment run on the Octet® instrument. Data shown is from 8 Streptavidin Biosensors with biotin-Protein A captured, binding human IgG and taken through regeneration with 8 different reagents over 11 binding cycles.

## 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 Octet® Analysis Studio 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 1.0, glycine pH 2.0 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.

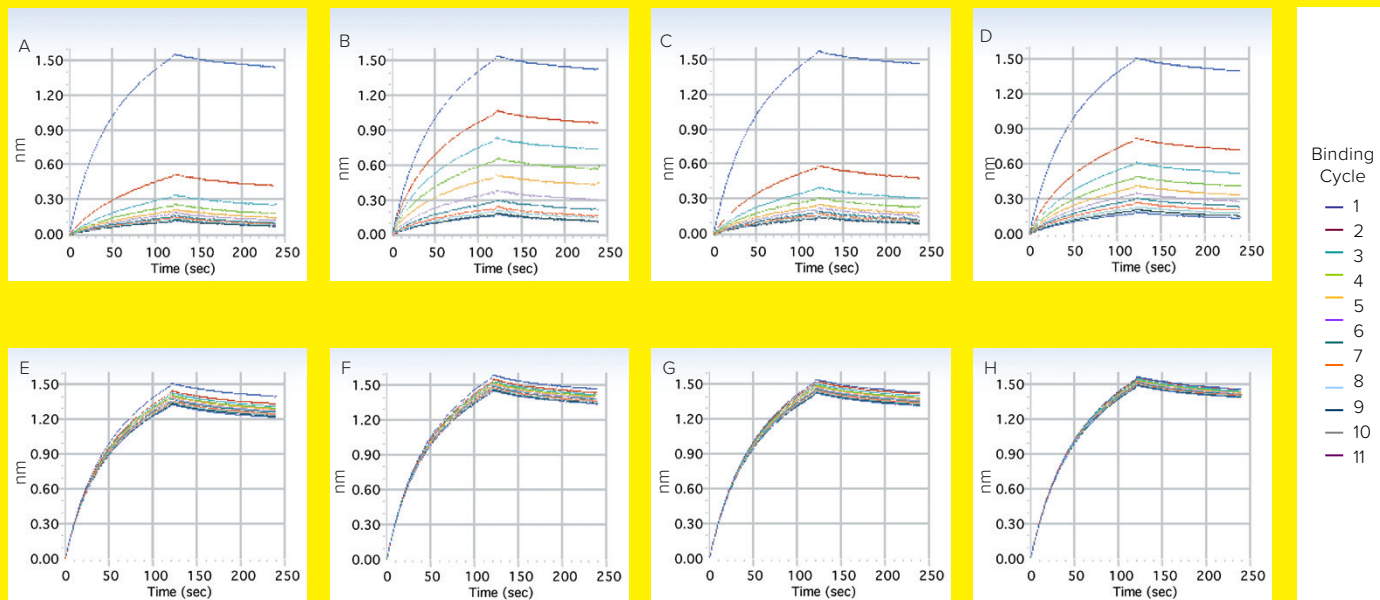


Figure 6: Analysis of effectiveness of eight different reagents in regenerating Protein A captured to Streptavidin Biosensors. Reagents tested were: A) 5.0 M NaCl; B) 0.01% SDS; C) NaOH, pH 10.0; D) NaOH, pH 11.0; E) HCl pH 0.5; F) 10 mM Glycine pH 1.0; G) 10 mM glycine, pH 2.0; H) 500 mM phosphoric acid

## Biosensor selection guide

|   | Anti-Human IgG Fc | Anti-Murine IgG Fc | Protein A | SA      | SSA     | AR   | APS | AHC |
|---|-------------------|--------------------|-----------|---------|---------|------|-----|-----|
| Application:<br>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

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