TECH NOTE

nicoya

Analysis of biotin-tagged proteins on Alto digital SPR using a streptavidin functionalized sensor

Overview

Nicoya's Streptavidin Kit allows users to irreversibly capture biotinylated ligands directionally, offering an optimal orientation for analyte binding. This method also enables users to capture ligands from crude samples or matrix compositions, which may be incompatible with direct coupling methods. This technical note examines the performance of the Streptavidin Kit with Alto™, Nicoya's digital surface plasmon resonance™ (SPR) instrument, to measure the kinetics of biotinylated IL-6R binding to an anti-IL-6R antibody Tocilizumab, an immunosuppressant. Alto's pre-optimized Streptavidin Kit and protocols allow users to reduce experiment design time by offering a pre-developed assay configuration.

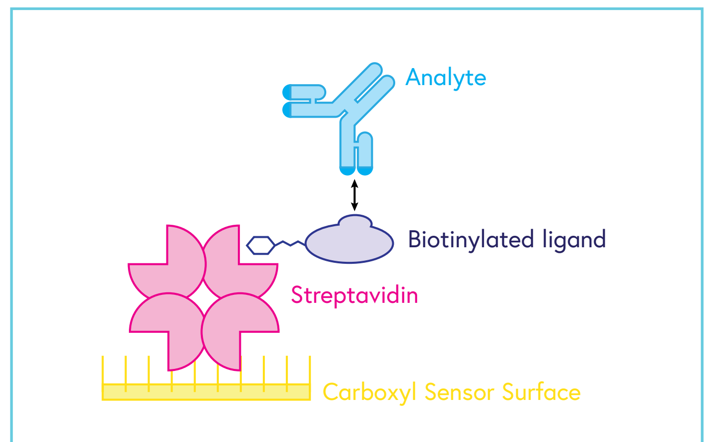

Figure 1: *Schematic representation of an assay using the Streptavidin Kit.*

Introduction

Streptavidin is a tetrameric protein found in the bacterium *Streptomyces avidinii*, widely known for its extremely high binding affinity for the molecule biotin (also known as Vitamin H). This affinity is on the order of 10^{-14} mol/L, which is 10^3 to 10^6 times tighter than an antibody-antigen interaction, and the strongest known non-covalent interaction between a protein and ligand.[1] Once formed, the biotin-avidin bond is resistant to a wide range of pH and temperatures, solvents and other harsh solutions. Streptavidin is used extensively in many biotechnology applications due to its extremely high affinity for biotin and overall stability.[2-5] These properties are leveraged in SPR for capturing biotinylated ligands.

The Streptavidin Kit is used by amine coupling the streptavidin to carboxyl sensors to create a streptavidin-functional surface that captures biotin-tagged ligands. The Streptavidin Kit is compatible with several assay types: kinetics, screening, and quantitation. For example, when using Alto's direct kinetics protocol, users may capture their biotinylated ligands onto streptavidin and measure kinetics between their analytes and the captured ligand (Figure 1).

Materials

Materials included in the Streptavidin Kit (ALTO-R-STV-KIT):

- 10x Streptavidin Aliquots (ALTO-R-STREPTAVIDIN)
- 1.5 mL 10 mM Sodium Acetate pH 5.0 (ALTO-R-IMB-5.0)

Other equipment & materials used:

- Alto 16-Channel Instrument with Nicosystem Pro Software (ALTO16)
- Alto 16-Channel Carboxyl Cartridge (KC-CBX-CMD-16)
- Running Buffer: PBS-T (0.1% Tween 20), pH 7.4 (ALTO-R-PBST)
- Alto Carboxyl Surfacing Kit: cleaning, normalization, activation (ALTO-R-CBX-SURF)
- Regeneration Buffer: 10 mM Glycine-HCl pH 3.0 (ALTO-R-GLYHCL-3.0)

- Recombinant Human IL-6R Protein (ECD, His & Avi Tag), Biotinylated, Sino Biological Cat# 10398-H49H-B
- Tocilizumab (anti-IL-6R), Selleckchem.com, Cat# A2012

Assay Optimization Tips

- The ligand may be either purified or in a crude matrix. For best performance, it is recommended that analytes be purified.
- For most applications, the user should choose the lowest ligand density that still provides an analyte binding signal, to prevent multiphasic behavior and other artefacts from oversaturating the sensor surface. It is recommended that the user choose a ligand density that gives a maximum analyte response (Rmax) between 50-150 RU to give a sufficient signal-to-noise ratio to resolve kinetics while avoiding steric hindrance or mass transfer effects.
- The Streptavidin Kit is compatible with a wide range of running buffers and buffer additives. Recommended buffers:
  - PBS-T (0.1% Tween 20), pH 7.4 (ALTO-R-PBST)
  - HBS-T (0.1% Tween 20), pH 7.4 (ALTO-R-HBST)
  - TBS-T (0.1% Tween 20), pH 7.4 (ALTO-R-TBST)

* *TBS-T not to be used in auxiliary buffer for capture molecule immobilization.*

- Optimizing buffer conditions to capture the biotinylated ligand is not necessary; it is recommended (but not required) that the ligand be in the running buffer.
- Streptavidin aliquots are single-use. Do not freeze-thaw or combine freeze-thawed aliquots with fresh aliquots.

Experiment Setup

The Streptavidin Kit is compatible with several assay types: direct kinetics, direct screening, and quantitation. Each protocol has a specific toggle labeled 'Biotin-Avidin Strategy', which the user needs to toggle 'ON' when using the Streptavidin Kit (Figure 2). For example, when using Alto's Direct Kinetics protocol with 'Biotin-Avidin Strategy' toggled ON, users may capture their biotinylated molecules onto streptavidin and measure kinetics between their analytes and the captured ligand. Streptavidin and biotin bind irreversibly, therefore the ligand will remain immobilized after surface regeneration.

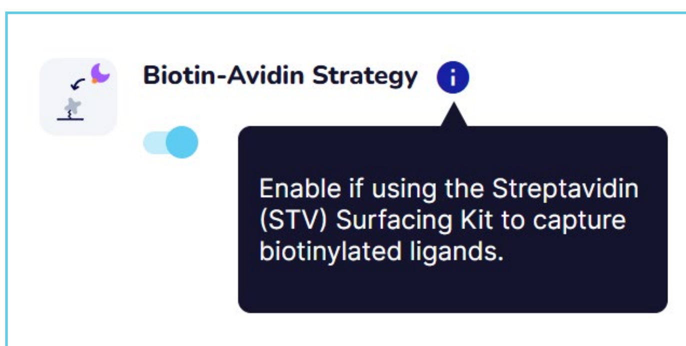

Figure 2: *Biotin-Avidin Strategy toggle. To capture biotinylated ligands with the Streptavidin Kit, the toggle must be turned on in a compatible experiment type (Direct Kinetics, Direct Screening or Quantitation).*

The experimental setup was remotely completed on Alto's User Portal, followed by run initiation on the instrument:

1. From a laptop, the experiment was designed and saved in the User Portal.
2. On the instrument, the designed method was selected to launch Alto's on-screen setup guide.
3. A carboxyl cartridge was placed in the instrument, and samples were loaded into the cartridge following the experiment setup guide.
4. The experiment was initiated on the Alto device by selecting "Run Method".

Sample Preparation

Diagrammatic representation for the preparation of 10 µg/mL Streptavidin aliquots is shown in Figure 3.

1. Retrieve the 4 µL Streptavidin aliquot (stock concentration is 0.4 mg/mL) provided with the Streptavidin Kit. Thaw the aliquot at room temperature prior to preparing the dilution.
2. Add 156 µL of 10 mM Sodium Acetate buffer pH 5.0 to the 4 µL aliquot of Streptavidin to create the final concentration of 10 µg/mL.
3. Mix the solution by pipetting up and down the Streptavidin sample before loading immediately into the cartridge.
4. Load 65 µL into well R6, and dispose of any excess solution.

Assay Protocol

The following steps were completed automatically by Alto with no operator supervision.

1. Carboxyl sensors were normalized with normalization solutions.
2. Carboxyl sensors were primed with 10 mM HCl for 60 s.
3. Carboxyl sensors were activated with 200 mM EDC/NHS for 600 s.
4. The streptavidin from the Streptavidin Kit diluted in 10 mM Sodium Acetate pH 5.0 was immobilized onto all sensors for 600 s.
5. All sensors were blocked with the 1 M ethanolamine for 300 s to quench any remaining active carboxyl groups.
6. All sensors were conditioned for 60s with 10 mM Gly-HCl pH 3.0.
7. 2 µg/mL samples of biotinylated IL-6R in the running buffer were introduced to each even-numbered active sensor for 300 s.
8. Alto executed five automated Tocilizumab serial dilutions on the cartridge. Each sample was diluted from 150 nM stock, producing 0.62 nM, 1.85 nM, 5.55 nM, 16.6 nM, and 50 nM solutions in the running buffer.
9. The lowest Tocilizumab concentration was exposed to each sensor for 180 s, followed by dissociation in the running buffer for 600 s, and a 60 s regeneration step with 10 mM Glycine-HCl pH 3.0.

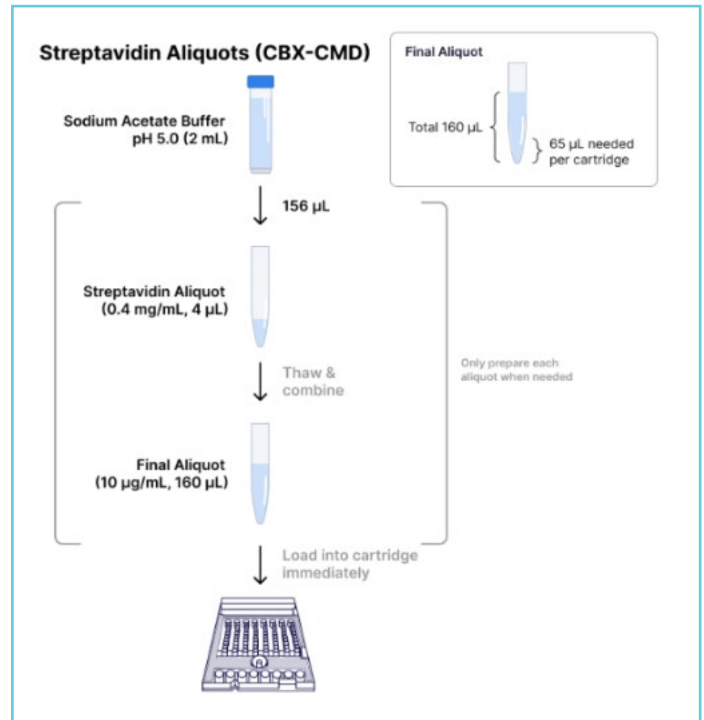

Figure 3: *Dilution instructions for the Streptavidin Kit.*

10. Step 9 was repeated for the remaining four Tocilizumab analyte concentrations, which constitutes a full multi-cycle kinetics (MCK) round.

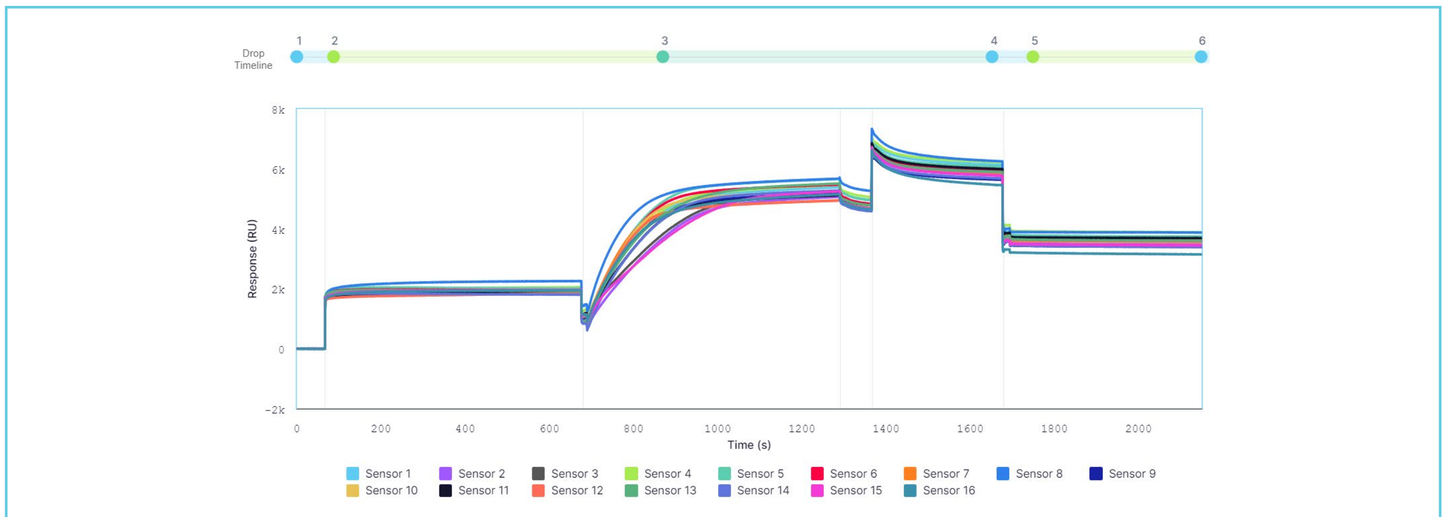

Figure 4: *Activation of the 16 channels with 200 mM EDC/NHS from Nicoya's Surfacing Kit, followed by immobilization of 10 µg/mL of streptavidin in 10 mM sodium acetate pH 5.0, which is further followed by blocking of the sensors with 1 M Ethanolamine. The image was generated by the Nicosystem Software.*

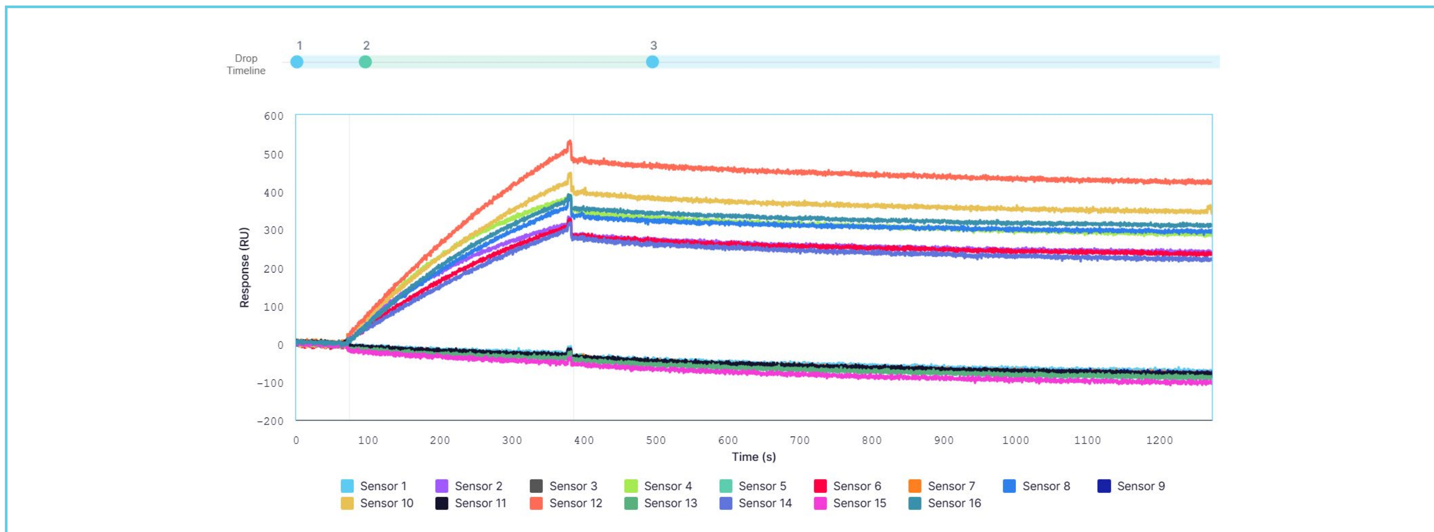

Figure 5: *Immobilization of 2 μg/mL biotinylated IL-6R in PBS-T to streptavidin functionalized surface. The image was generated by the Nicosystem Software.*

Data Analysis

1. Open the test under the analysis tab in the portal.
2. Check the build avidin surface tab and assess streptavidin immobilization levels across all 16 sensors in the cartridge to ensure that response falls in the normal range of 3000-4000 RU.
3. Check the biotin ligand immobilization tab and assess ligand immobilization across the 8 active channels in the cartridge to ensure sufficient and/or optimal levels for kinetics.
4. Click the Direct Kinetics tab. A 1:1 Langmuir binding model will be automatically applied to the data.
5. Use processing tools as required.
6. Download final images and/or .CSV files.

Results & Discussion

The capability of streptavidin for use as a capture surface in SPR assays was evaluated. For each experiment, streptavidin was immobilized onto both the reference and response sensors of the cartridge. Figure 4 shows an immobilization overlay for a cartridge used as part of this study, with an average immobilization level of 3607 RU for the streptavidin. Figure 5 shows an immobilization overlay for the binding of biotin-tagged IL-6R to streptavidin. Table 1 summarizes the average immobilization level and error for both of these immobilization steps.

To test the ability of streptavidin to be used as a functional surface for kinetics determination, single-cycle kinetics (SCK) assays using Tocilizumab binding to an immobilized biotin-IL-6R were performed. Complete regeneration of the analyte was achieved with 10 mM Glycine-HCl, pH 3.0, demonstrating the reusability of the sensor surface (Figure 6). This figure also highlights the low susceptibility of NSB to the streptavidin surface as evidenced in the lack of response in the reference channel for the Tocilizumab analyte.

	Streptavidin (10 μg/mL)	**biotin-IL-6R (2 ug/mL)**
Average (RU)	3607	364
Std Dev (RU)	187	68.6

Table 1: *Streptavidin and biotin-IL-6R immobilization % CV.*

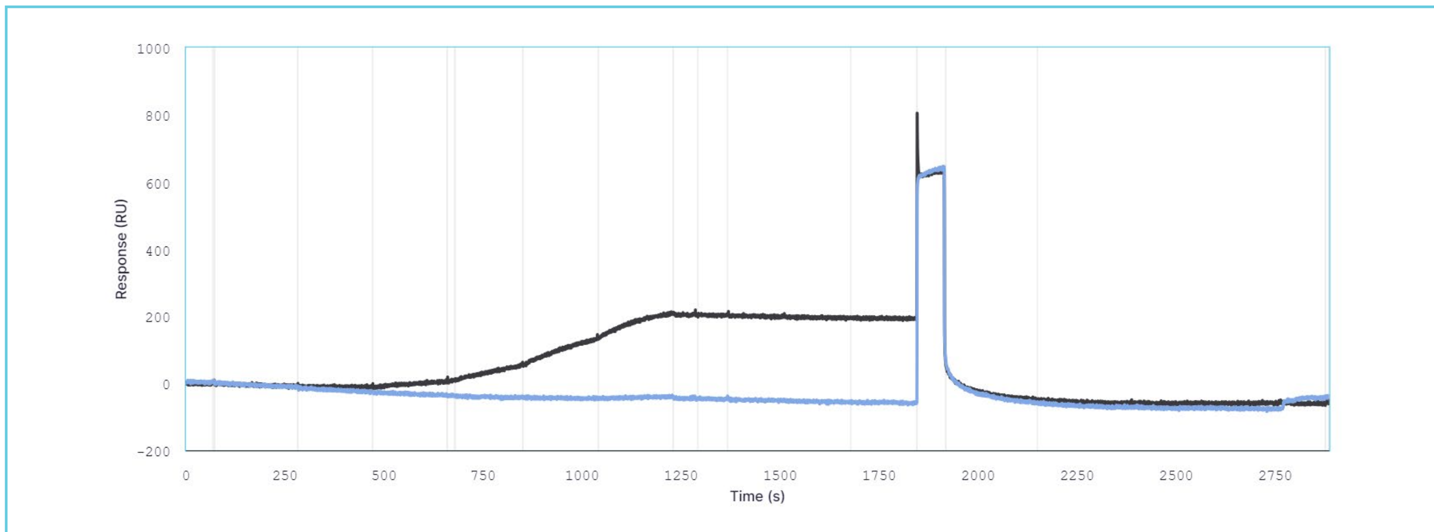

Figure 6: *Reference (blue trace) and active channel (black trace) binding data showing binding of Tocilizumab to the biotin-IL-6R analyte for each analyte concentration. Following the dissociation of each analyte concentration, full regeneration was achieved by 10 mM Glycine-HCl pH 3.0.*

Kinetic values for SCK kinetics were calculated based on the sensorgrams obtained on one cartridge with Alto, tested across all 8 lanes and 2 rounds. A representative example of a sensorgram is shown in Figure 7. The data were fit to a Langmuir 1:1 binding model analyzed in the Nicosystem Software. Excellent reproducibility was demonstrated across all channels and rounds. From the kinetic analysis, association and dissociation rate constants when the Streptavidin Kit was used in an SCK format were determined to be $1.43 \times 10^5 \pm 4.20 \times 10^4$ $M^{-1}s^{-1}$ and $4.08 \times 10^{-5} \pm 3.10 \times 10^{-5}$ s^{-1}, respectively, resulting in a KD of 267 ± 178 pM.

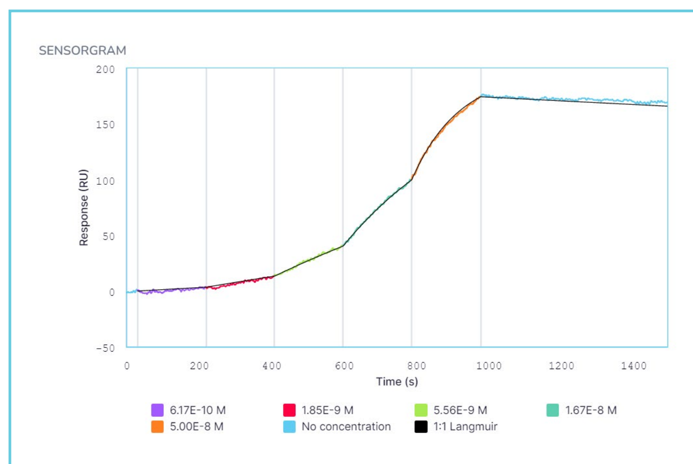

Figure 7: *Single-cycle kinetics of Tocilizumab (analyte) binding to immobilized biotin-IL-6R (ligand) on Alto. The analyte was titrated from 0.62 nM to 50 nM. Black curves are the Langmuir 1:1 binding fit model analyzed in the Nicosystem Software.*

Regeneration

Surface regeneration in SPR involves the removal of bound analytes from the sensor surface and restoring it to its analyte-free state for subsequent analyses. The choice of regeneration solution must be optimized for each specific interaction to ensure all analyte is removed and the ligand still retains full binding activity.

The Streptavidin Kit is compatible with many regeneration solutions due to the stability of streptavidin and the high affinity of the biotin-streptavidin interaction. Regeneration will not remove the biotinylated ligand from the surface, but it can destroy the activity of the ligand. An ideal regeneration solution is strong enough to completely remove the analyte, but not harsh enough to damage the biotinylated ligand. As shown in Figure 6, the regeneration step with 10 mM glycine-HCl pH 3.0 results in a sharp change in response that returns to the same baseline position as before the analyte association step. This is indicative of a successful regeneration.

Recommended regeneration solutions are available in the Regeneration Optimization Kit (ALTO-R-REGEN-OPT), for those who wish to find the best reagent that suits their binding interaction.

References

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