TECH NOTE

Analysis of HIS-tagged ligands on Alto digital SPR using an anti-HIS capture sensor

Overview

Nicoya's Anti-HIS Capture Kit gives users the ability to capture histidine-tagged (HIS-tagged) ligands directionally, offering an optimal orientation for analyte binding. This method also enables users to capture HIS-tagged ligands from crude samples, or from matrix compositions which may be incompatible with direct coupling methods. This technical note examines the performance of the Anti-HIS Capture Kit with Alto™, Nicoya's digital surface plasmon resonance™ (SPR) instrument, to measure the kinetics of HIS-tagged Influenza A H5N1 hemagglutinin (HA) antigen and a H5N1 HA antibody. Alto's pre-optimized Anti-HIS Capture Kit and protocols allow users to cut down on experiment design time by offering a pre-developed assay configuration.

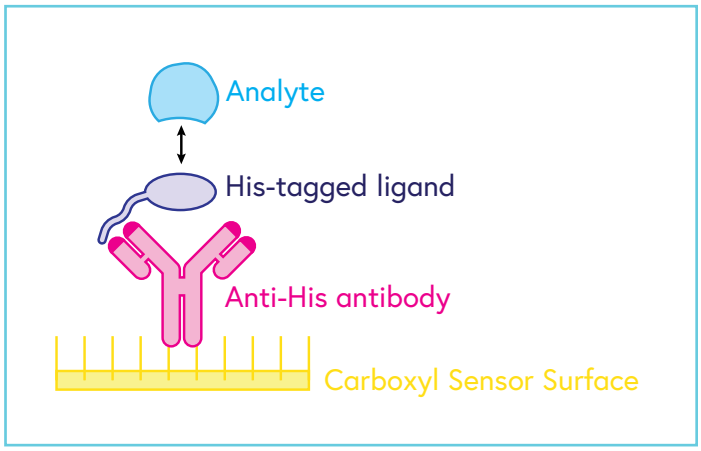

Figure 1: *Schematic representation of an assay using the Anti-HIS Capture Kit.*

Introduction

Anti-HIS antibodies are antibodies that specifically recognize and bind to histidine-tagged proteins. A histidine tag typically consists of six to ten histidine residues added to either the N- or C-terminus of the protein of interest. In molecular biology and biochemistry, histidine (HIS)-tags are commonly added to recombinant proteins to facilitate their purification and detection[1]. With Alto's Anti-HIS Capture Kit, the HIS tags can further be used to tether ligands to the sensor surface and perform kinetic analyses.

Alto's Anti-HIS Capture Kit contains enough anti-HIS antibody and immobilization buffer for ten experiments. Other materials needed for use of the Anti-HIS Kit include the Alto Carboxyl Cartridge and Surfacing Kit, both of which are available for purchase.

The Anti-HIS Capture Kit is used by amine coupling the anti-HIS antibody to carboxyl sensors. Using Alto's capture kinetics protocol, users may then capture their HIS-tagged molecules onto the immobilized antibody and measure kinetics between their analytes and the captured ligand.

Materials

- Alto 16-Channel Instrument with Nicosystem Pro Software (ALTO16)
- Alto 16-Channel Carboxyl Cartridge (KC-CBX-CMD-16)
- Running Buffer: PBS-T (0.1% Tween20), pH 7.4 (ALTO-R-PBST)
- Regeneration Solution: 10 mM Glycine-HCl, pH 1.5
- Alto Carboxyl Surfacing Kit: cleaning, normalization, activation (ALTO-R-CBX-SURF)
- Anti-HIS Capture Kit (ALTO-R-HIS-KIT)
- Recombinant Influenza A H5N1 Hemagglutinin / HA Protein: Sino Biological, CAT#: 11048-V08H1
- Influenza A Virus Hemagglutinin / HA Antibody, Rabbit mAb: Sino Biological, CAT#: 86001-RM02

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 Anti-HIS Capture 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 biotin ligand is not necessary; it is recommended (but not required) that the ligand be in the running buffer.
- Anti-HIS antibody aliquots are single-use. Do not freeze-thaw or combine freeze-thawed aliquots with fresh aliquots.

Experiment Setup

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. An Alto 16-Channel 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

Preparation of 5 µg/mL anti-HIS Aliquots

1. Retrieve an anti-HIS aliquot (0.2mg/mL, 4 µL) and allow it to equilibrate to room temperature.
2. Using a pipette, add 156 µL of 10 mM Sodium Acetate buffer pH 4.0 to the initial aliquot to create the final aliquot solution (5 µg/mL, 160 µL).
3. Mix the solution by pipetting up and down.
4. Immediately load 3 µL of 5 µg/mL anti-HIS solution into Wells C1 -> C8 of the cartridge for capture kinetics or load 65 µL of 5 µg/mL anti-HIS solution into Well R6 for capture screening. Dispose of any leftover solution.

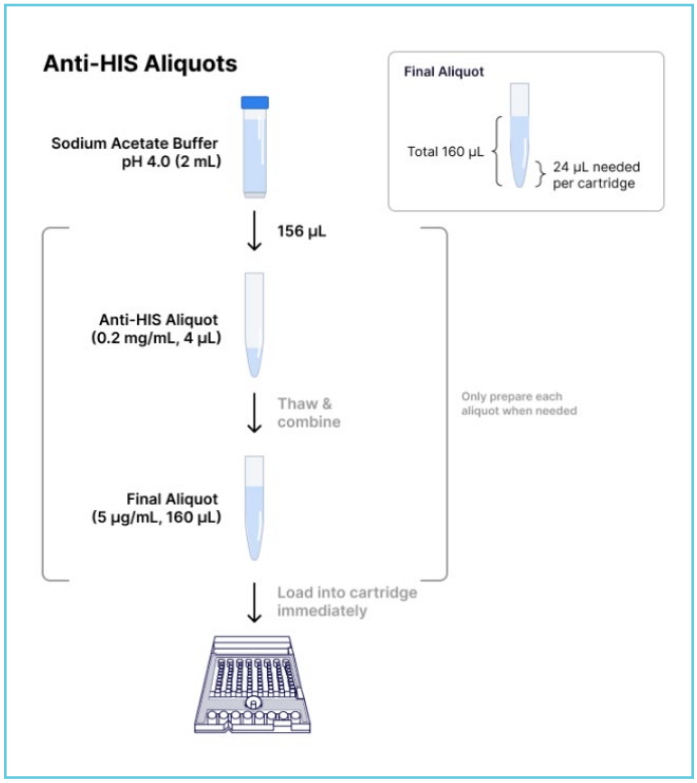

Figure 2: *Dilution instructions for the Anti-HIS Capture Kit.*

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 anti-HIS antibody from the Anti-HIS Capture 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. 5 µg/mL samples of HIS-tagged H5N1 HA in the running buffer were introduced to each even-numbered active sensor for 300 s.
8. Alto executed five automated anti-H5N1 HA antibody RMO2 serial dilutions on the cartridge. Each sample was diluted from 300 nM stock, producing 1.23 nM, 3.70 nM, 11.1 nM, 33.3 nM, and 100 nM solutions in the running buffer.
9. The lowest RM02 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 1.5..
10. Step 9 was repeated for the remaining four RM02 analyte concentrations, which constitutes a full multi-cycle kinetics MCK round.

Data Analysis

1. Open the test under the analysis tab in the portal.
2. Check the build capture surface tab and assess anti-HIS immobilization across all 16 sensors in the cartridge to ensure sufficient and/or optimal levels.
3. Click the Capture Kinetics tab. A 1:1 Langmuir binding model will be automatically applied to the data.
4. Use processing tools as required.
5. Download final images and/or .CSV files.

Results & Discussion

The capability of the anti-HIS antibody was evaluated for use as a capture reagent in SPR assays. For each experiment completed, the anti-HIS antibody was immobilized onto both the reference and response sensors of the cartridge. Figure 3 shows a sample antibody immobilization overlay for a cartridge used as part of this study, with an average immobilization level of 3281 RU for

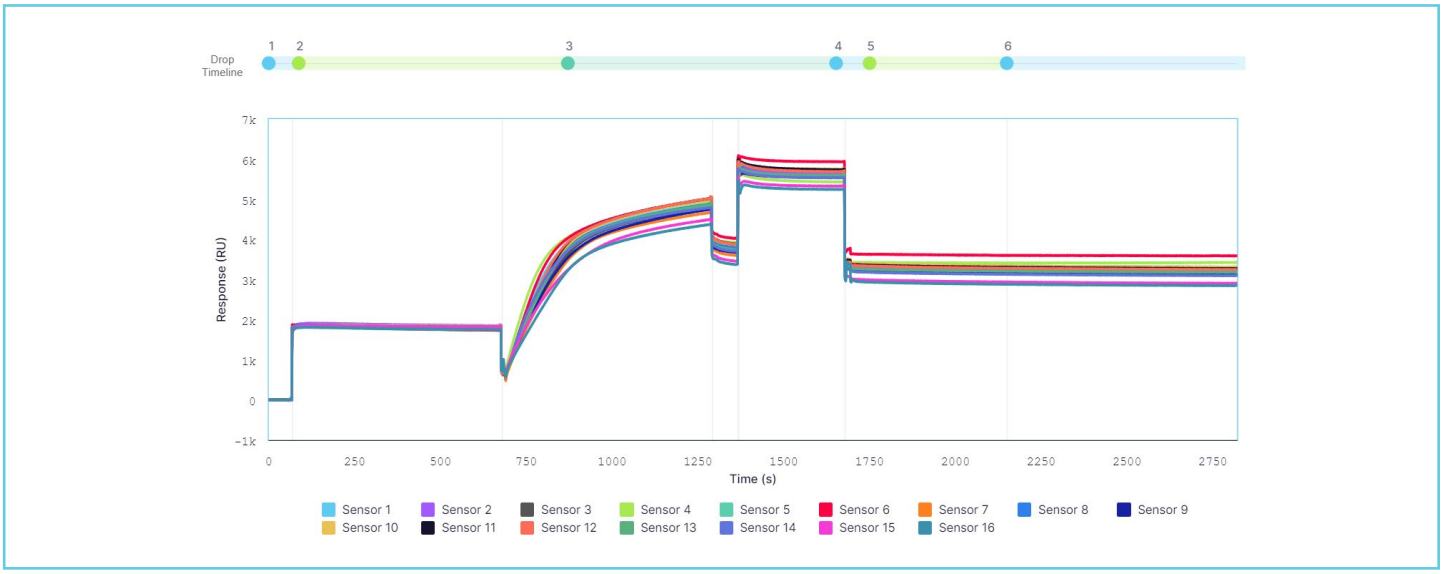

Figure 3: *Immobilization of anti-HIS antibody on 16 sensors simultaneously on the carboxyl cartridge. The capture surface building activity includes activation of all 16 channels with 200 mM EDC/NHS from Nicoya's Carboxyl Surfacing Kit for 600 s, followed by immobilization of 5 µg/mL anti-HIS antibody in sodium acetate pH 4.5 for 900 s, and blocking of sensors with 1 M Ethanolamine for 300 s. The immobilization level of the anti-HIS antibody was 3281 RU.*

the anti-HIS antibody. The immobilization level may be adjusted, if necessary, by changing the contact time or concentration of the anti-HIS antibody.

	Anti-HIS antibody immobilization (RU)	HIS-tagged H5N1 HA Capture (RU)
Average	3281	215.0
Std Dev	165.5	53.76

Table 2: *Anti-HIS antibody immobilization and capture of HIS-tagged H5N1 HA*

To test the ability of anti-HIS antibody immobilized sensors to be used as a capture surface for kinetics determination, MCK assays using an HIS-tagged HA ligand binding to an anti-HA antibody were performed. In the MCK format, the recapture of the HIS-tagged HA ligand and MCK binding of the anti-HA antibody analyte was consistent across all 8 lanes for one cycle, as shown in the top overlay plot of Figure 4. This overlay also demonstrates the low susceptibility of NSB to the anti-HIS antibody surface as evidenced in the lack of response in the reference channel for the anti-HA antibody analyte. Complete regeneration of the ligand and bound analyte was achieved with 10 mM Glycine-HCl, pH 1.5, demonstrating the reusability of the sensor surface. The HIS-tagged HA ligand shows minimal dissociation following binding to the anti-HIS antibody surface, highlighting the stability of this capture system.

Kinetic values for the MCK assay were calculated based on the sensorgrams obtained on one cartridge with Alto, tested across 8 lanes and 2 rounds. The reference data was subtracted from the response for each sensor to generate sensorgrams which were fit to a Langmuir 1:1 binding model analyzed in the Nicosystem Software. Kinetic parameters obtained for the Influenza A H5N1 HA antigen and a H5N1 HA antibody interaction using the anti-HIS antibody capture molecule is reported in Table 3 and demonstrates excellent reproducibility across all channels and rounds. From the kinetic analysis, association and dissociation rate constants were determined to be $7.50x10^{6} \pm 1.64x10^{5}$ $M^{-1}s^{-1}$ and $2.05x10^{-4} \pm 4.18x10^{-5}$ s^{-1}, respectively, resulting in a K_D of 288 ± 95.7 pM (Figure 5).

Regeneration

Surface regeneration in SPR involves the removal of non-covalently bound reagents from the sensor and restoring it for subsequent analyses. The choice of regeneration solution must be optimized for each specific interaction.

The Anti-HIS Capture Kit is compatible with many regeneration solutions. An ideal regeneration solution is strong enough to completely remove the ligand and analyte, but not so harsh as to damage the capture surface. As shown in Figure 4, each regeneration step results in a sharp change in signal that returns it to the same baseline position as before the analyte association step. Additionally, each capture cycle throughout the assay reached a similar binding level, indicating both a full regeneration, and lack of damage to the capture

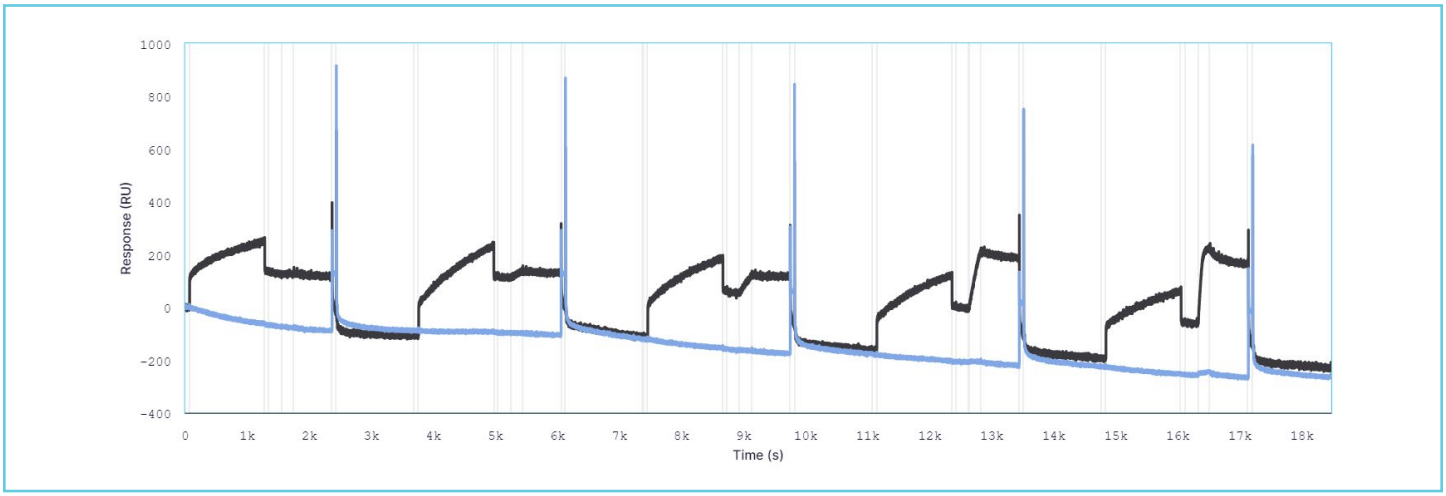

Figure 4: *The anti-HIS antibody immobilized sensors provide reproducible multi cycle kinetics results and can be efficiently regenerated several times. 5 µg/mL HIS-tagged HA was captured on an anti-HIS antibody sensor (drop in response due to bulk shift) followed by sequential association of the anti-HA antibody analytes at concentrations of 412 pM, 1.2 nM, 3.7 nM, 11.1 nM and 33.3 nM. The sensor surface was regenerated with a 60 second exposure of 10 mM Glycine-HCl, pH 1.5 after the dissociation phase.*

k_a (1/M*s)	k_d (1/s)	K_D (pM)
$7.50x10^{6} \pm 1.64x10^{5}$	$2.05x10^{-4} \pm 4.18x10^{-5}$	288 ± 95.7

Table 3: *Kinetic parameters measured for anti-HA antibody binding to HIS-tagged HA captured by the anti-HIS antibody capture molecule.*

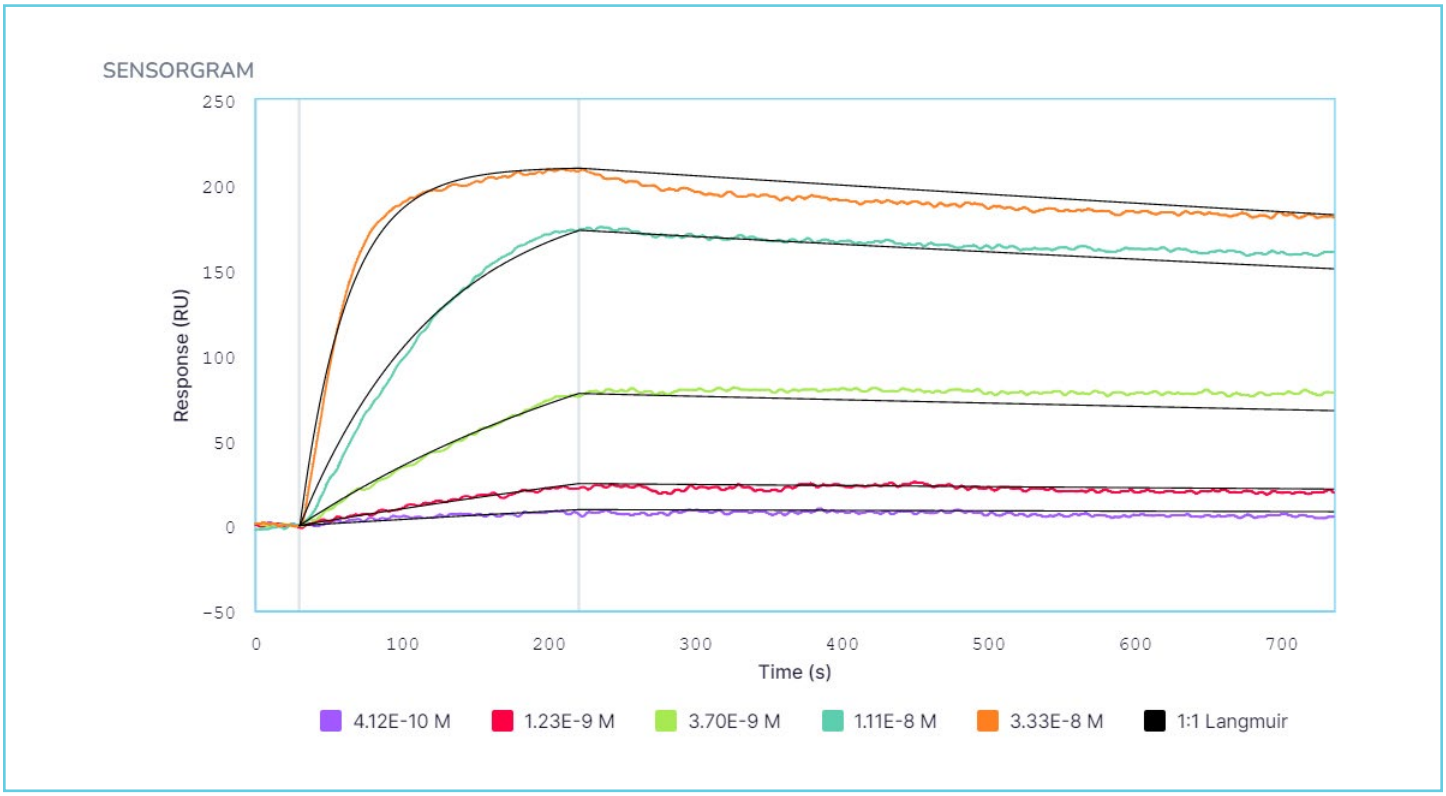

Figure 5: *Sample sensorgrams showing the binding of anti-HA antibody to HIS-tagged HA that is captured by anti-HIS antibody in an MCK format. Image was generated by the Nicosystem Software.*

surface. 10 mM Glycine-HCl, pH 1.5 (ALTO-R-GLYHCl-1.5) was the optimal regeneration solution determined. It is recommended that 10 mM Glycine-HCl, pH 1.5 always be used as the regeneration solution for the Anti-HIS Capture Kit, since the anti-HIS antibody is always what will be regenerated, but there are several other regeneration solutions 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

1. Hochuli E, Bannwarth W, Döbeli H, Gentz R, Stüber D. Genetic Approach to Facilitate Purification of Recombinant Proteins with a Novel Metal Chelate Adsorbent. Bio/Technology. 1988; 6(11): 1321–5.