Gator Anti-His (HIS) XT Probes

For Quantitation, Kinetic Characterization and Epitope Binning of His-tagged Proteins

Product Information

Product Name: Gator® Anti-His (HIS) XT Probes

Product No: 160050

Description: A tray of 96 Gator Anti-His (HIS) XT Probes coated with an Anti-His NovoBody™ for high-performance His-tagged protein capture, for use in Q/K/QKR/EP assays

Key Features

- **First-of-its-kind AI-designed NovoBody™ capture protein** integrated into a ready-to-use biosensor surface for His-tagged protein analysis
- **High affinity His-tag binding** (k_{off} 10^{-5}-$10^{-6}s^{-1}$) delivers superior baseline stability with reduced signal drift to measure picomolar affinities reliably (K_D < 100 pM) with minimal ligand dissociation artifacts
- **Low sample consumption** of His-tagged proteins required for capture, providing cost savings
- **Advanced XT signal resolution** provides reproducible performance for samples including challenging small (<15 kDa) or low affinity analytes
- **Wide dynamic range** for fast and accurate quantitation without dilution (0.2 - 1000 µg/mL)
- **Highly regenerable surface** enables 10x reuse with minimal capacity loss
- **Low nonspecific binding** ensures clean, reliable ligand capture in complex samples without unwanted Fc-mediated interactions
- **10-fold faster loading** for increased throughput

Overview

Gator HIS XT Probes are the first to incorporate a novel AI-designed anti-His NovoBody™ (designed by Monod Bio) as a ready-to-use probe, delivering next-generation BLI performance for His-tagged protein analysis. This probe is engineered to enable industry-leading high affinity specific poly-His capture with far greater capture half-life, providing a robust platform for real-time quantitation or kinetic analysis and epitope binning with interacting analytes.

The HIS XT Probe capture surface exhibits high affinity and specificity toward both N- and C-terminal His-tags, ensuring stable ligand capture with 58% less baseline drift compared to other anti-His capture systems. With an advanced optical design, HIS XT delivers up to 4-fold higher response per captured protein along with low non-specific binding. Together, HIS XT enables reliable measurements for analytes with pM - µM affinities in complex media, inclusive of small analytes (<15 kDa). Its high dynamic range enables optimized avidity control and flexibility as well as simplified quantitation workflows.

With low sample requirements, 10-fold faster loading compared to anti-His biosensors and robust regeneration (>10 cycles with >90% capacity retention), HIS XT Probes provide a cost-effective solution for applications including kinetic characterization, high throughput screening, titer analysis and epitope binning and mapping.

Kinetic Assay Workflow

HIS XT Probes are suited for robust kinetic analysis due to their high ligand loading capacity and exceptionally stable baseline performance. The NovoBody-based capture surface exhibits equal sensitivity toward both N-terminal and C-terminal His tags while enabling rapid capture characterized by fast on-rates.

In most cases, **~1–5 µg/mL** His-tagged protein is sufficient to load the ligand onto the sensor and perform kinetic characterization with its binding partners.

When performing regeneration, the captured His-tagged protein will be removed with the analyte and will require to be re-captured prior to the analysis of the next analyte sample.

Example Kinetics Protocol:

1. **Pre-wet:** Assay buffer 180–600 s, 1000 rpm
2. **Baseline: A**ssay buffer 120 s, 1000 rpm
3. **Loading:** His-tagged capture protein 10-60 s, 1000 rpm
4. **Baseline:** Assay buffer 30-120 s, 1000 rpm
5. **Association:** Analyte 60–600 s, 1000 rpm
6. **Dissociation:** Assay buffer 60–600 s, 1000 rpm
7. **Regeneration:** Regen Buffer pH 2.0 (No Salt) 5 s, 1000 rpm
8. **Neutralization:** Assay buffer 5 s, 1000 rpm

Repeat steps 2-8 for additional analyte samples.

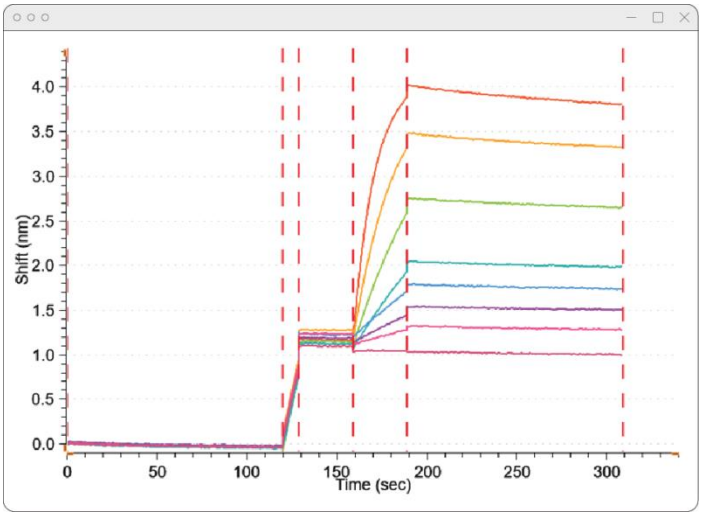

Figure 1: Representative kinetics (K) assay sensorgram showing capture of His-tagged CD64 on the His XT probe, followed by association and dissociation of human IgG1.

Quantitation Assay Workflow

HIS XT Probes support both one-step and two-step quantitation workflows. In one-step quantitation, His-tagged analyte in unknown samples binds directly to the HIS XT probe and is quantified against a standard curve. In two-step quantitation, a His-tagged capture protein is first loaded onto the probe, followed by measurement of analyte binding to the captured protein. Both methods can be analyzed using endpoint or rate-based probe response to concentration.

- **High specificity:** Selectively recognizes His-tags across a wide range of recombinant proteins
- **Broad quantitation range:** 0.2–1000 µg/mL
- **Minimal interference from crude media**
- **Recommended regeneration:** Regen Buffer pH 2.0 (No Salt) (PN:120008)

Standard Curve Preparation:

Prepare a serial dilution series of purified His-tagged protein (one-step) or target analyte (two-step) in the same matrix as your samples. For example:

- 1000 µg/mL → 0.122 µg/mL in a 2-fold serial dilution, 14 points
- Adjust range based on expected sample concentrations

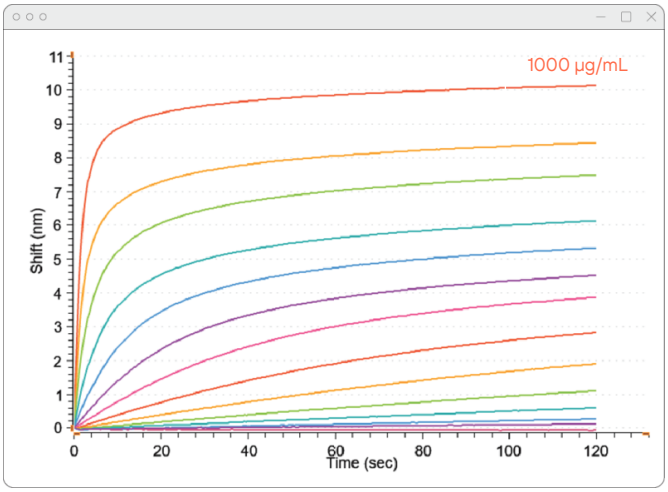

Figure 2: His-HSA standard curve using 2-fold dilution series from 1000 µg/mL to 0.24 µg/mL measured using the HIS XT probe with 120 sec acquisition time at 400 rpm.

One-Step Quantitation Example Protocol:

1. **Pre-wet:** Assay buffer 60-120 s, 400 rpm
2. **Quantitation:** Sample or Standard 30-60 s, 400 rpm
3. **Regeneration:** Regen Buffer pH 2.0 (No Salt) 5 s, 1000 rpm
4. **Neutralization:** Assay buffer 5 s, 1000 rpm

Repeat steps 2-4 for all samples and standards.

Two-Step Quantitation Example Protocol:

Note: To build this protocol select Assay Setup > K Assay

1. **Pre-wet:** Assay buffer 60-120 s, 400 rpm
2. **Baseline:** Assay buffer 120 s, 1000 rpm
3. **Loading:** His-tagged capture protein 10-30 s, 1000 rpm
4. **Baseline:** Assay buffer 30 s, 400 rpm
5. **Quantitation:** Sample or Standard 30-60 s, 400 rpm
6. **Regeneration:** Regen Buffer pH 2.0 (No Salt) 5 s, 1000 rpm
7. **Neutralization:** Assay buffer 5 s, 1000 rpm

Repeat steps 2-7 for all samples and standards.

Epitope Binning Workflow

The HIS XT probes high loading capacity, stable baseline, and regeneration robustness make it ideal for tandem epitope binning workflows. In tandem binning, the His-tagged antigen is captured on HIS XT probes, followed by sequential exposure to two antibodies. Based on the binding behavior, epitope classification of competitive (same bin) or non-competitive (different bins) can be mapped. Complete characterization of 256 antibody pairs can be performed in under 4 hours using the Gator Plus system.

Recommended his-tagged antigen concentration: 1 µg/mL - 10 µg/mL.

Tandem Epitope Binning Example Protocol:

1. **Pre-wet**: Assay buffer 600 s, 1000 rpm
2. **Baseline**: Assay buffer 30 s, 1000 rpm
3. **Loading**: His-tagged capture protein 10 – 60 s, 1000 rpm
4. **Baseline**: Assay buffer 30 s, 1000 rpm
5. **Association 1**: mAb 1 160 – 180 s, 1000 rpm
6. **Association 2**: mAb 2 60 – 180 s, 1000 rpm
7. **Regeneration:** Regen Buffer pH 2.0 (No Salt) 5 s, 1000 rpm
8. **Neutralization:** Assay buffer 5 s, 1000 rpm

Repeat steps 2-8 for all samples.

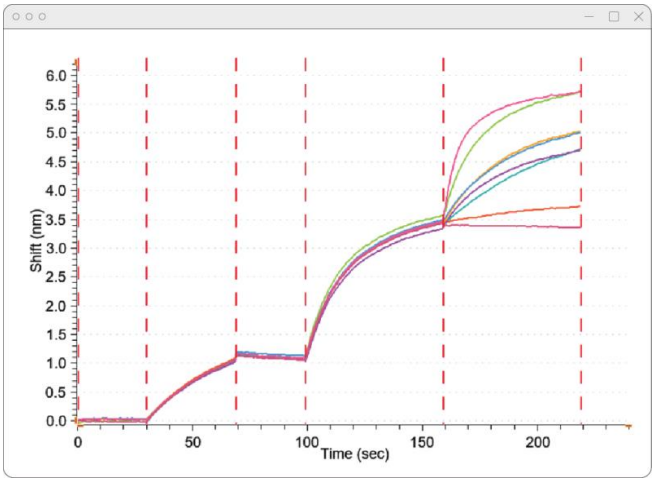

Figure 3. Representative sensorgram of a tandem epitope binning experiment.

Regeneration

HIS XT Probes demonstrate robust regeneration performance due to their NovoBody-based capture surface, which is inherently more stable than conventional antibody probes. This enhanced stability results in minimal loss of surface activity following repeated regeneration cycles, ensuring consistent performance across multiple assays.

Regen Buffer pH 2.0 (No Salt) is recommended to use for regeneration for 10+ cycles for most applications. Optimal regeneration conditions may vary depending on the ligand and analyte pair. It is recommended to verify that regeneration fully removes bound material while maintaining capture performance across cycles. Do not use low-pH, high-salt buffers with HIS XT probes.

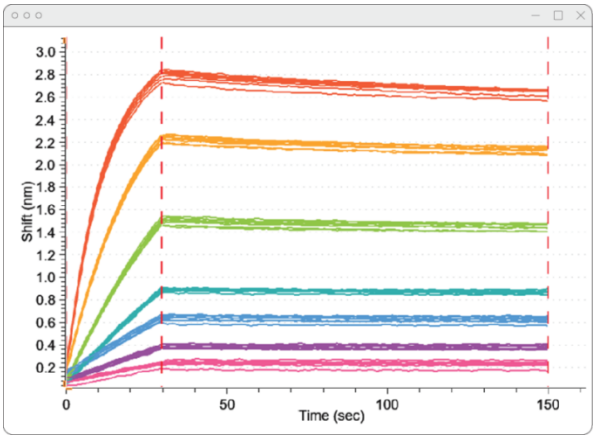

Figure 4: Re-capture of His-CD64 protein binding to human IgG1 over 10 cycles from HIS XT probes after regeneration using Regen Buffer pH 2.0 (No Salt) ≤ 5% CV of baseline recovery is maintained through each recapture cycle across all concentrations.

Best Practices

Control Loading Density

Avoid saturating the probe surface with ligand. Lower loading levels reduce mass-transport limitations and prevent rebinding artifacts during dissociation. Target 0.5-1.0 nm for most applications.

Optimize for Analyte Size

- **Large or multivalent analytes (>100 kDa, IgG, multimers):** Reduce loading concentration/time, decrease temperature to 25°C, and use 400 rpm to minimize avidity effects
- **Small analytes (<15 kDa, peptides, fragments):** Increase loading concentration to 50-100 nM or extend loading time to improve signal resolution

Match Sample Matrix

Prepare standards in the same buffer as your samples (e.g., culture media, lysate) to account for refractive index differences and reduce nonspecific binding artifacts. Equilibrate probes in sample matrix before starting the assay.

Implement Reference Subtraction

Include reference sensors (no ligand loaded) and buffer-only wells to subtract background signal and instrument drift from specific binding measurements.

Validate Regeneration

Verify that regeneration fully removes bound material while maintaining capture performance across cycles. Test regeneration by running a reference standard after each cycle. Do not use low-pH, high-salt buffers with HIS XT probes.

Ordering Information

Product Name	Package	Product Number
Gator® Anti-His (HIS) XT Probes	Tray of 96 probes	160050
Regen Buffer pH 2.0 (No Salt)	50 mL bottle	120008
BLI 96 - Flat Black Plate	10 pack	130150
BLI 96 - Flat Black Plate	100 pack	130260
K Buffer	1000 mL	120072
K Buffer	50 mL	120011
Q Buffer	1000 mL	120071
Q Buffer	50 mL	120010

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