

His Probes

Catalog No. 160009

Overview

Gator™ His Probes are used to capture polyhistidine-tagged (His-tagged) proteins for quantitation (Q), kinetics (K) and quantitation-kinetics-regeneration (QKR) assays. His probes come pre-coated with a high-affinity, monoclonal antibody that recognizes His-tags on both the C and N-termini. These probes can be used to measure purified samples in buffer as well as proteins in supernatant or crude mixtures.

Materials Required

His Probes	Catalog No. 160009
Max Plate	Catalog No. 130062
Black Plates	Greiner 655209
Quantitation (Q) Buffer	Catalog No. 120010
Kinetics (K) Buffer	Catalog No. 120011
Regeneration Buffer	Catalog No. 120012

Storage

Store at room temperature in the foil pouch, ensuring zipper is fully sealed to avoid humidity/moisture contamination. In high-humidity environments, storage inside a dry cabinet is recommended.

General Applications

1. Quantitation of His-tagged protein
2. Kinetics assay of His-tagged protein
3. Determination of concentration and kinetics of interaction of His-tagged protein in one run (QKR)

GENERAL METHODS

Sample Volume

Black Plate: 200 μ L (180 μ L minimum)
 Max Plate: 250 μ L (280 μ L maximum)

Pre-wet Conditions

250 μ L assay buffer (Q or K) in Max Plate,
 5 min at 1000 rpm

Regeneration to Save on Consumables

His probes can be regenerated using the Gator™ software. (Settings are in Assay Setup). Regeneration buffer and neutralization buffer (Q or K Buffer) should be placed in adjacent wells in either the Black Plate or the Max Plate. For His probes, 3 cycles of 5 seconds for regeneration is recommended. Regeneration before assay is recommended to ensure run-to-run consistency. After regeneration, probes can be stored in assay buffer and kept at 4°C for >2 weeks.
Note: The probe can be regenerated up to 10 times when used with purified samples. Number of regenerations with crudes samples will be sample dependant.

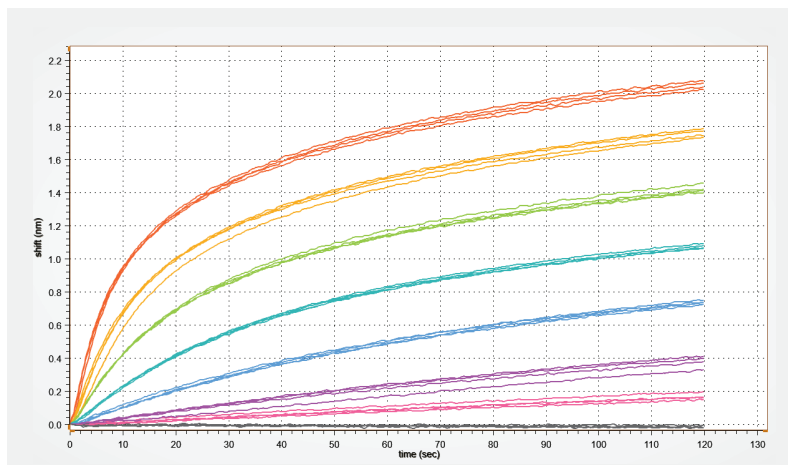


Figure 1: Repeated measurements of His-tagged human serum albumin (His-HSA; 0 to 500 μ g/mL in Q Buffer) on the same His Probe following regeneration. %CV is <5% for all concentrations.

Rapid Quantitation of Unknown Samples

Quantitation of crude and purified unknown samples can be performed using the Q assay preset on the Gator™ software. For accurate results, make a standard curve of known concentrations in the same buffer as the unknowns. For the most accurate quantitation results, fresh probes are recommended.

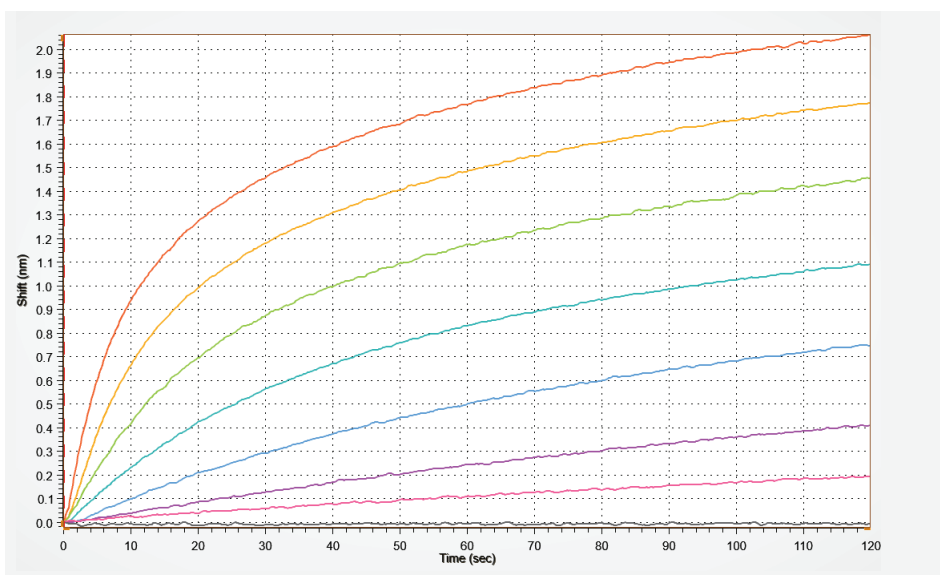


Figure 2: Standard curve of His-HSA (12.5 – 500 μ g/mL in Q Buffer) bindings to His probe. Assay was performed at 400 rpm for 120 sec.

Kinetics Study of His-tagged Proteins with Binding Partners

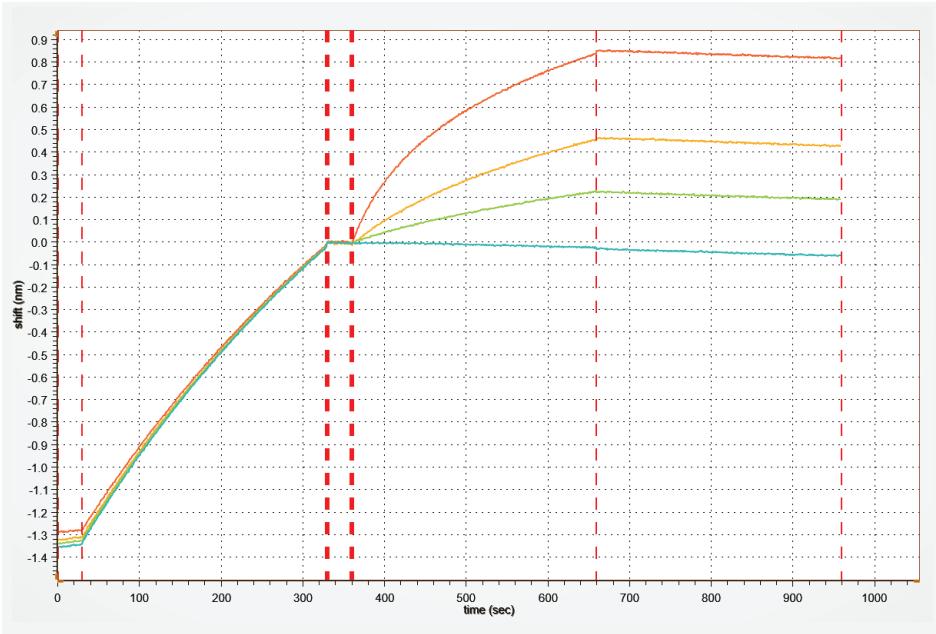


Figure 3: Loading of His-LAG3 (100 nM diluted in K Buffer) onto His probes followed by association and dissociation of FGL1 at various concentrations (0 to 300 nM in K Buffer). Global-fit analysis found the affinity to be 8 nM.

