Gator™ Ni-NTA Kit

Catalog No.350002

OVERVIEW

Gator™ Ni-NTA probes are designed for quantitation and kinetic characterization of His-tagged biomolecules in crude or purified samples. The ready to use probes are manufactured with a high affinity and high specificity Ni-NTA from QIAGEN which is pre-immobilized on a Gator™ Probe. The specially designed regeneration and neutralization buffers for Ni-NTA probe eliminate the need for a separate recharging step which dramatically reduced assay time and significantly simplified the assay workflow. In conjunction with the GatorPrime and GatorPlus, the Ni-NTA probes provide a rapid single step regeneration protocol including recharging for quantitation and kinetics. Better baseline stability enhanced the kinetics and off-rate ranking assay performance. With the easy and rapid regeneration capability, the Ni-NTA probes are also perfectly suited for epitope binning, serving as an alternative to Gator™ Anti-His probes.

MATERIALS REQUIRED

Ni-NTA Kit	Catalog No.350002
Max Plate	Catalog No. 130062
Black Plate	Greiner 655209 (96 well) Greiner 781209(384 well)
Quantitation Buffer	Catalog No. 120010
Kinetics Buffer	Catalog No. 120011

STORAGE

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

ASSAY PRINCIPLE AND GENERAL APPLICATIONS

- The Ni-NTA quantitation and kinetics assays are based on the strong binding of Ni^{2+} to the His-tagged biomolecules
- Epitope binning assay is applicable using the single step regeneration protocol including recharging
- Determination of concentration and kinetics of interaction of His-tagged protein in one run (QKR)
- Concentrations of experimental samples are calculated based on their binding rate compared to that of the known concentrations that make up the standard curve
- The Ni-NTA ligand used in Gator™ Ni-NTA probes is based on the high affinity and high specificity ligands developed from QIAGEN
- The generic Gator™ Ni-NTA probes in combination with Gator™ system provides high quality total titer information and kinetic data
- Ni-NTA probes can be regenerated for up to 20 times with the single step regeneration protocol including recharging in 30 seconds for various applications such as quantitation, kinetics, off-rate ranking and epitope binning. Different from other vendor which must be recharged with Ni^{2+} prior to loading the next His-containing proteins for the regenerated biosensor, the newly designed
- Gator™ Ni-NTA regeneration buffer has simplified the recharging and regeneration process in single step regeneration protocol

TYPICAL PERFORMANCE OF QUANTITATION

- Dynamic range: 0.25-1000 µg/mL (Q buffer); 1-1000 µg/mL (media diluted 10x in Q buffer)
- Throughput: 8 samples in 2 minutes, 96 samples in 34 minutes
- Precision/accuracy: < 10% CVs
- Up to 20 regenerations can be achieved using purified protein in Q buffer

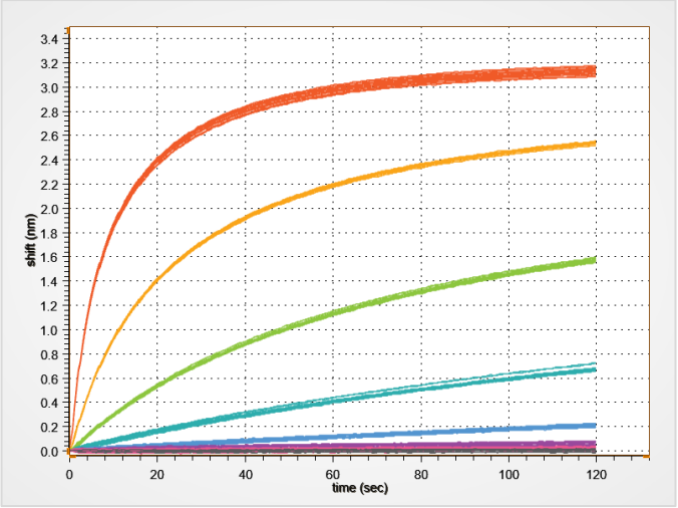

Figure 1: Quantitation of His-Protein A in quantitation buffer after 20 regeneration cycles. Data was acquired in 120s at 1000 rpm.

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GENERAL METHODS

Sample Volume

- Black Plate (96 well plate): 200 µL (180 µL minimum)
- Black Plate (384 well plate): 100 µL (80 µL minimum)

Pre-wet Conditions

- 250 µL of buffer diluent (280 µL maximum) in Max Plate, 10 min at 1000 rpm

REGENERATION

The underlying surface chemistry of the Gator™ Ni-NTA probe is robust and stable over a broad range of pH. Most His-tagged protein interactions binding to the Ni-NTA probes can be disrupted using new designed regeneration buffer. The ideal regeneration condition for the Ni-NTA probe is 5 sec each at 1000 rpm in Gator™ Ni-NTA Regeneration Buffer and Ni-NTA Neutralization Buffer (provided in Ni-NTA Kit) for a total of three cycles. The probes can be reused in buffer diluent up to 20 times without showing significant loss of binding to His-tagged protein. Optimization of regeneration conditions is recommended for each unique His-tagged protein and assay buffer condition.

TIPS FOR QUANTITATION ASSAYS

- Pre-hydrate the Gator™ Ni-NTA probes for at least 10 minutes in assay buffer or matrix that is an exact match to the sample being analyzed. This will minimize background response from non-specific binding to the biosensor
- Always include a blank when generating standard curves or running unknowns. This will be used as a reference for background subtraction
- during data analysis
- For accurate results, a standard curve must be generated using the same His-tagged protein as the sample(s) to be quantitated.
- The standards should be diluted in a buffer matrix that is an exact match of the unknown sample(s)
- Standard curves can be saved and utilized for subsequent experiments, if the biosensors are from the same manufacturing lot as those used to run the samples
- The concentration of sample(s) being analyzed should fall within the concentration range of the standard curve for accurate quantitation

KINETIC ASSAY GENERAL WORKFLOW

Step Number	Step	Time (Sec)	Shake Speed (rpm)
1	Baseline	60	1000
2	Loading	180	1000
3	Baseline	60	1000
4	Association	300	1000
5	Dissociation	600	1000
6	Regeneration	5	1000
7	Neutralization	5	1000
8	Regeneration	5	1000
9	Neutralization	5	1000
10	Regeneration	5	1000
11	Neutralization	5	1000

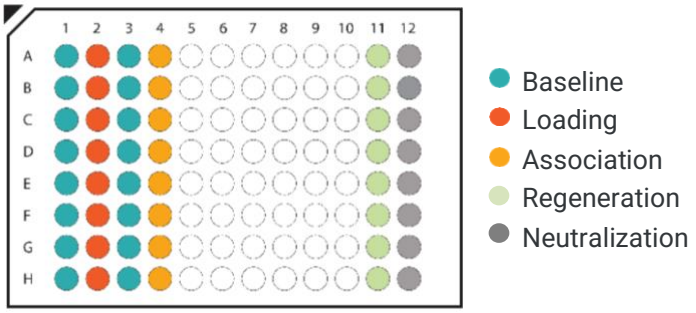

Figure 2: Example of a kinetic assay plate map on Black Plate (96 well)

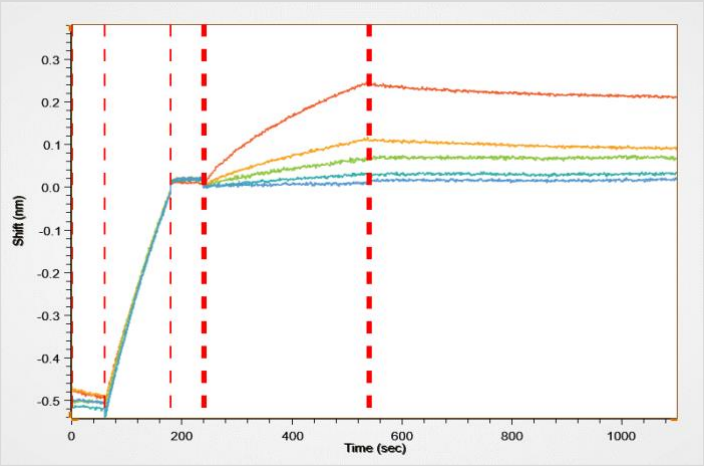

Figure 3: Kinetic assay of CD64 and human IgG using Ni-NTA in kinetics buffer (1000rpm at 30°C). Significantly more stable baselines were achieved compared to Gator™ Anti-His probes (data not shown).

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REGENERATION

The ideal regeneration condition for the Ni-NTA probe is 5 sec each at 1000 rpm in regeneration and neutralization buffers for a total of three cycles. The probes can be reused in buffer diluent up to 10 times without significant variation of the kinetic binding parameters. Optimization of regeneration conditions is recommended for each unique His-tagged protein and assay buffer condition.

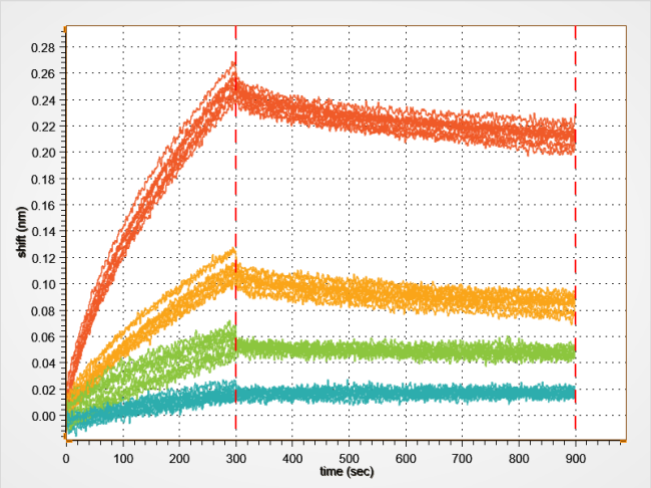

Figure 4: Association and dissociation of human IgG to His-CD4 after 10 regeneration cycles.

	k_{off}(1/s)	k_{on}(1/Ms)	K_D(M)
1	2.54E-04	1.39E+04	1.83E-08
2	3.34E-04	2.25E+04	1.48E-08
3	3.25E-04	1.39E+04	2.34E-08
4	2.78E-04	2.00E+04	1.39E-08
5	3.05E-04	1.78E+04	1.71E-08
6	2.72E-04	1.66E+04	1.64E-08
7	2.58E-04	1.59E+04	1.62E-08
8	2.68E-04	1.48E+04	1.81E-08
9	2.90E-04	1.78E+04	1.63E-08
10	3.04E-04	2.17E+04	1.40E-08

Table 1: Kinetic analysis of the His-CD64 and human IgG interaction using the same Ni-NTA probes with the single step regeneration protocol including recharging, after 10 regeneration cycles. Kinetics buffer was used for all assay steps and the assay temperature was 30°C.

TIPS FOR KINETIC ASSAYS

- Pre-hydrate the Ni-NTA probes for at least 10 minutes in assay buffer or matrix that is an exact match to the sample being analyzed. This will minimize background response from non-specific binding to the biosensor
- Always include a reference well that has no ligand for accurate baseline subtraction
- For accurate results, the loading on the His-tagged protein should be optimized and should not exceed 1 nm. Slower loading speed (eg. 400 rpm) can help to improve immobilization of the His-tagged protein on the probe
- The His-tagged protein and the binding partner should be diluted in same assay buffer
- Dedicate a separate reference well to check for non-specific binding of the ligand to the probe

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