PRODUCT INSERT

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. The superior Gator® Ni-NTA Regen Buffer (PN: 120052) and Neutral Buffer (PN: 120053) eliminate the need for a separate recharging step, dramatically reducing assay time and simplifying the assay workflow. In conjunction with the Gator® system, the Ni-NTA probes provide a rapid single step regeneration protocol including recharging for quantitation and kinetics. Ni-NTA probes provide better baseline stability which enhanced the kinetics and off-rate ranking assay performance. With the easy and rapid regeneration capability, the Ni-NTA probes are also a great fit for epitope binning, serving as an alternative to Gator® Anti-His probes.

MATERIALS REQUIRED

Gator® Ni-NTA Kit	Catalog No. 350002
Max Plate	Catalog No. 130062
Black Plate	Greiner 655209 (96 well)
	Greiner 781209 (384 well)
Q Buffer	Catalog No. 120010
K Buffer	Catalog No. 120011

* includes Ni-NTA Probes (PN: 160016), Ni-NTA Regen Buffer (PN: 120052) and Ni-NTA Neutral Buffer (PN: 120053)

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.

TYPICAL PERFORMANCE OF QUANTITATION

- Dynamic range: 0.25-1000 µg/mL (Q Buffer); 1-1000 µg/mL (media diluted 10x in Q Buffer)
- Time to Result: 8 samples in 4 minutes, 96 samples in 26 minutes
- Up to 20 regenerations can be achieved using purified protein in Q Buffer

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

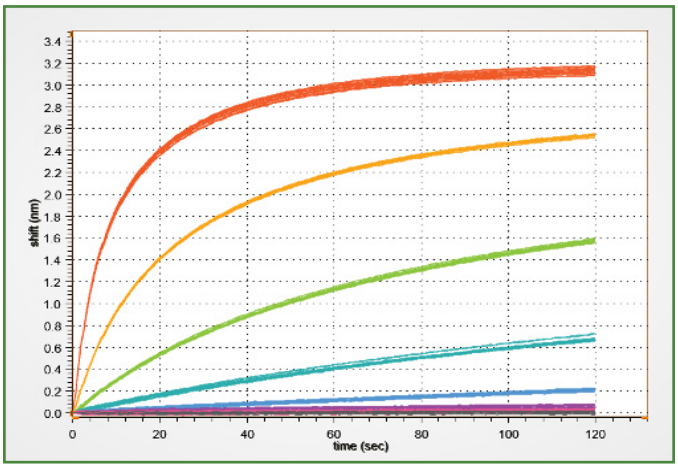

Figure 1: Binding curve of his-tagged protein A to Ni-NTA probes with 3 fold dilution from ... ug/mL to ... ug/mL. Each color of the curves are the overlay of binding curves from 20 regenerations.

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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 unknown 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 on 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
- The single step regeneration protocol including recharging in 30 seconds for various applications such as quantitation and epitope binning. This is different from other vendors which must be recharged with Ni^{2+} prior to loading the next His-containing proteins for the regenerated biosensor
- Gator® Ni-NTA Regen Buffer and Neutral Buffer have simplified the recharging and regeneration process in single step regeneration protocol

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

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TIPS FOR KINETICS 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 analyte 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 theprobe
- 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

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

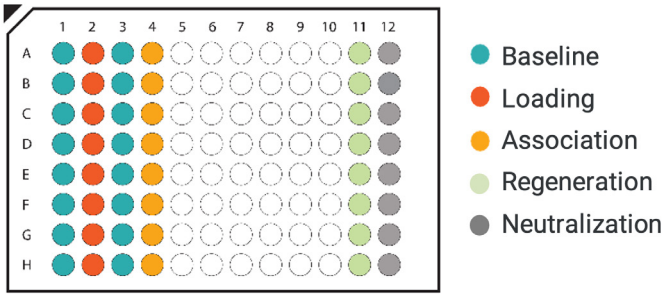

Figure 2: Example of a kinetics assay plate map on Black Plate (96 well).

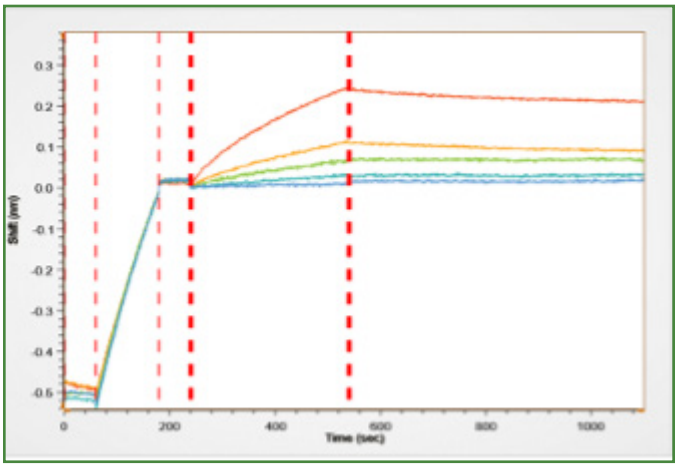

Figure 3: Kinetic assay of His-tagged CD64 and human IgG using Ni-NTA in K Buffer (1000 rpm at 30°C).

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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 the newly designed regeneration and neutralization buffers. The ideal regeneration condition for the Ni-NTA probe is 5 sec each at 1000 rpm in Gator® Ni-NTA Regen Buffer and Ni-NTA Neutral Buffer (provided in the Ni-NTA Kit) for a total of three cycles. The probes can be reused in buffer diluent up to 10 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.

Column1	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: Kinetics analysis of the His-tagged CD64 and human IgG interaction using the same Ni-NTA probes with the single step regeneration protocol including recharging, after 10 regeneration cycles. K Buffer was used for all assay steps and the assay temperature was 30°C.

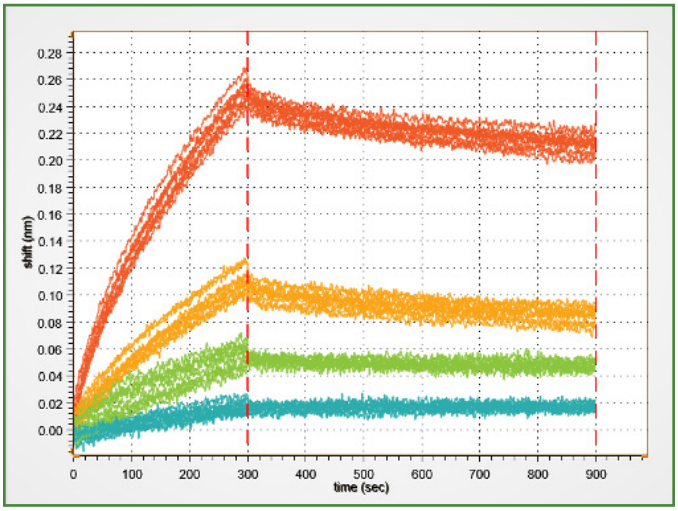

Figure 4: Association and dissociation of His-tagged CD64 and human IgG after 10 regeneration cycles.

ORDER ONLINE BELOW:

www.gatorbio.com

PN: 350002- Gator® Ni-NTA Kit
PN: 160016 - Gator® Ni-NTA Probes
PN: 120052 - Gator® Ni-NTA Regen Buffer
PN: 120053 - Gator® Ni-NTA Neutral Buffer

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