

## Gator™ Ni-NTA Kit for Quantitation and Kinetic Analysis of His-tagged Proteins

### Introduction

Addition of a polyhistidine tag (His-Tag) to the N- or C-terminus of proteins is commonly used for immobilized metal ion affinity chromatography (IMAC) purification of target proteins. The Gator™ Ni-NTA probes allow for rapid and continuous quantification of His-tagged proteins without the need for Ni<sup>2+</sup> recharging. The stable immobilization of His-tagged proteins allows for facile kinetic analyses with binding partners, off-rate screening, and epitope binning of antibodies experiments.

### Gator™ Ni-NTA Probe

Gator Ni-NTA probes are functionalized with Qiagen™ Tris-NTA and charged with Ni<sup>2+</sup> ions for high affinity immobilization of His-tagged proteins. The probes can be used for quantitation of His-tagged proteins in sample buffer and cell culture supernatants. The specially formulated regeneration and neutralization buffers allow for continuous regeneration of the probes, without the need for an additional Ni<sup>2+</sup> recharging step.



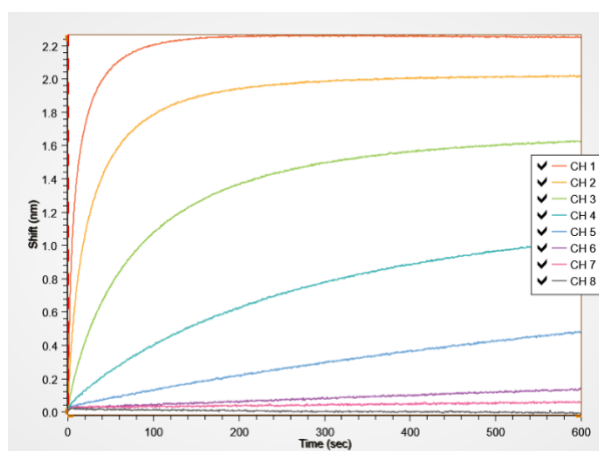
### Performance Summary

- Dynamic range: 0.25 – 1000 ug/mL (in Q buffer); 1 – 1000 ug/mL (in diluted cell culture media)
- Throughput: 8 samples in 2 minutes, 96 samples in 34 minutes
- Limit of detection: 0.25 ug/mL (10 min, 1500 rpm)
- Crude sample tolerant
- Cost effective - Reusable at least 20 times by regeneration in Q buffer; 10 times in diluted cell culture media

### Results

#### Dynamic range:

The dynamic range of Ni-NTA probes was tested using His-tagged Protein A diluted in Q buffer. The data was acquired in 10 min at 1,500rpm.



Known Conc. (ug/mL)	Calculated Conc. (ug/mL)	Binding Rate
1000	1018.307	0.3109
250	250.37	0.1038
62.5	65.731	0.0266
15.625	15.457	0.0051
3.906	4.05	0.0011
0.977	1.081	0.0003
0.244	0.25	0.0001

## Gator™ Ni-NTA Kit for Quantitation and Kinetic Analysis of His-tagged Proteins

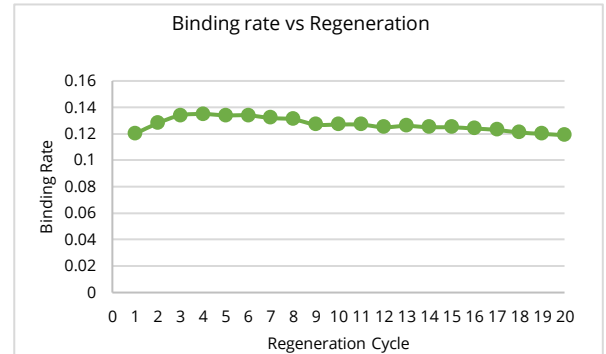
### Regeneration performance

#### Quantitation

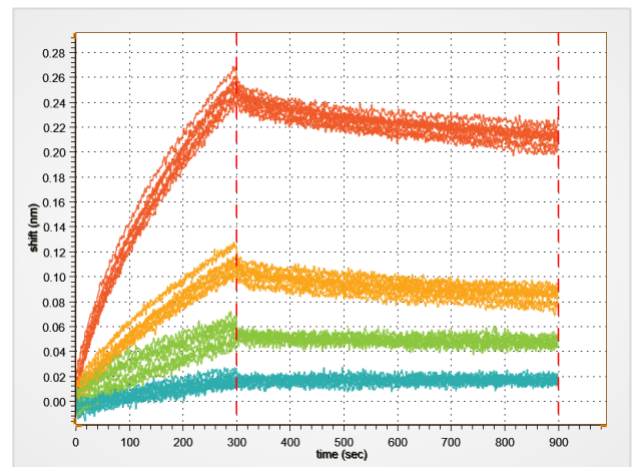
Figure on the right shows the quantitation performance after 20 regenerations of the same probe. No loss in binding rate observed even after 20 regenerations.

#### Kinetics

Similar kinetics parameters ( $k_{on}$ ,  $k_{off}$ ,  $K_D$ ) of the binding between human IgG with CD64 were obtained following 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

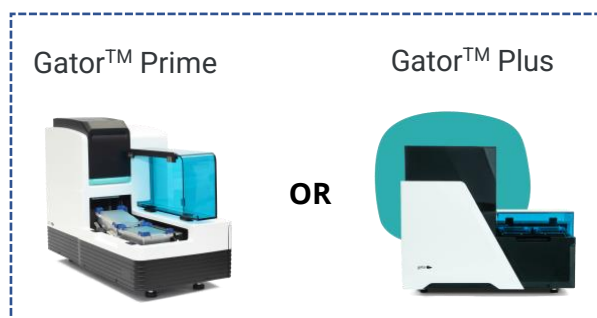


### Gator Bio Total Solution

Gator™ Ni-NTA Kit

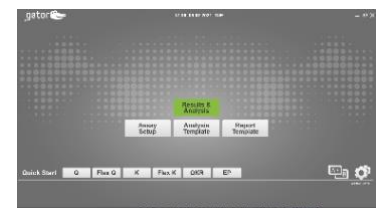


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GatorOne Software



### Ordering info:

PL168-350002: Gator™ Ni-NTA Kit