

Gator® Ni-NTA Kit for Quantitation and Kinetics Analysis of His-tagged Proteins

INTRODUCTION

Polyhistidine tag (His-Tag) on the N- or C-terminus of proteins is commonly used for immobilized metal ion affinity chromatography (IMAC). The Gator® Ni-NTA probes allow for rapid and continuous quantification of His-tagged proteins without the need for Ni^{2+} recharging step by using Gator® Ni-NTA Regen Buffer (PN: 120052) and Ni-NTA Neutral Buffer (PN: 120053). 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 PROBES

Gator® Ni-NTA probes are functionalized with Qiagen™ Tris-NTA and charged with Ni^{2+} 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 Gator® Ni-NTA Regen and Ni-NTA Neutral Buffers allow for continuous regeneration of the probes, without the need for an additional Ni^{2+} recharging step.

PERFORMANCE SUMMARY

- Dynamic range: 0.25–1000 $\mu\text{g/mL}$ (in Q buffer); 1–1000 $\mu\text{g/mL}$ (in diluted cell culture media)
- Time to Result: 8 samples in 4 minutes, 96 samples in 26 minutes
- LoD: 0.25 $\mu\text{g/mL}$ (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 1500 rpm

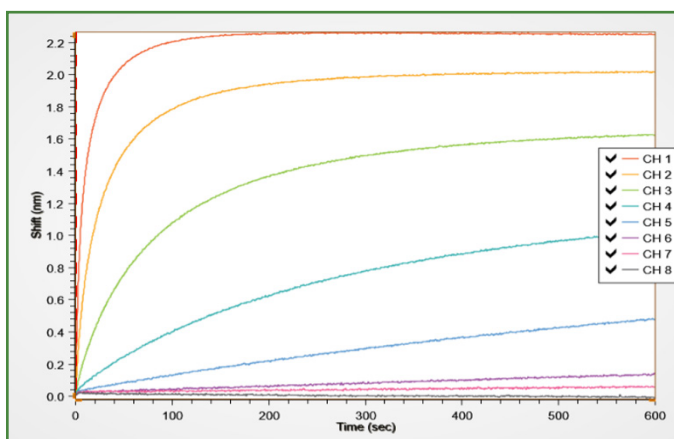


Figure 1: Detection of His-tagged Protein A standards using Ni-NTA probes.

Known Conc. ($\mu\text{g/mL}$)	Calculated Conc. ($\mu\text{g/mL}$)	Binding Rate
1000.00	1018.31	0.3109
250.00	250.37	0.1038
62.50	65.73	0.0266
15.63	15.46	0.0051
3.91	4.05	0.0011
0.98	1.08	0.0003
0.24	0.25	0.0001

Table 1: Calculated concentration and binding rate for the data from Figure 1.



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Quantitation

Figure 2 shows the quantitation performance after 20 regenerations from the detection of same concentration of His-tagged Protein A using Ni-NTA probes. No loss in binding rate observed even after 20 regenerations.

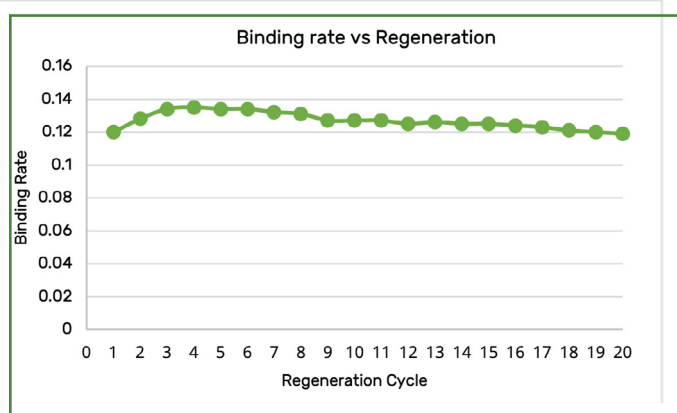


Figure 2: Binding rate results through 20 regeneration cycles from the detection of His-tagged Protein A using Ni-NTA probes. Regeneration before the assay is recommended practice.

Kinetics

Similar kinetics parameters (k_{on} , k_{off} , K_D) of the binding between His-tagged CD64 with human IgG 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

Table 2 : Kinetics results for the interaction between His-tagged CD64 with human IgG using Ni-NTA probes through 10 regenerations from Figure 3.

PRODUCT INFORMATION

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

*includes a set of Ni-NTA Probes, Ni-NTA Regen Buffer, Ni-NTA Neutral Buffer

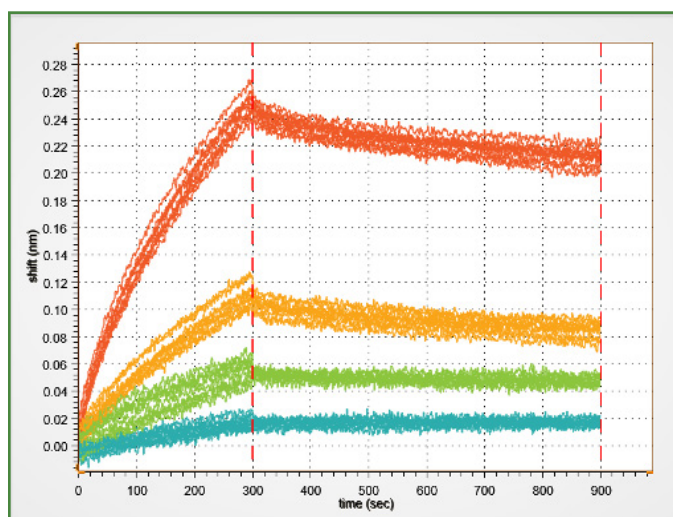


Figure 3: Kinetics interaction between His-tagged CD64 with human IgG through 10 regenerations. Regeneration before the assay is recommended practice.