

Gator® Next Generation HFC Probes

Introduction

Gator® Anti-Human IgG FC Gen II (HFCII) probes are high-performance nanobody-based biosensors that can detect and quantitate human IgG isotypes in different buffers (Q and K Buffer, Gator Bio Inc., PN: 120010, 120011), cell culture supernatant, cell media, and other crude samples.

The enhanced kinetic sensitivity, higher dynamic range for quantification, and better regeneration capabilities make these probes highly desirable in high-throughput applications such as epitope mapping/binning, lead-to-hit discovery and optimization, and cell line development.

Performance Summary

- Dynamic range: 0.3 - 6000 µg/mL in Q buffer; 1 - 2000 µg/mL in diluted cell culture media
- LoD: 0.1 µg/mL
- Crude sample tolerant
- Time to Result: 8 samples in 2 min, 96 samples in 34 min
- Cost-effective: Reusable at least 20 times in Q, K buffer and diluted cell culture media

Regeneration Performance

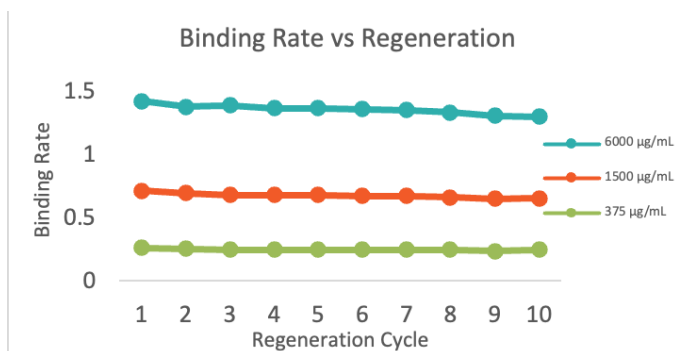


Figure 2: Binding rate of human IgG (6000, 1500, and 375 µg/mL) to HFCII. No loss of binding was observed after 10 regeneration cycles.

Quantification

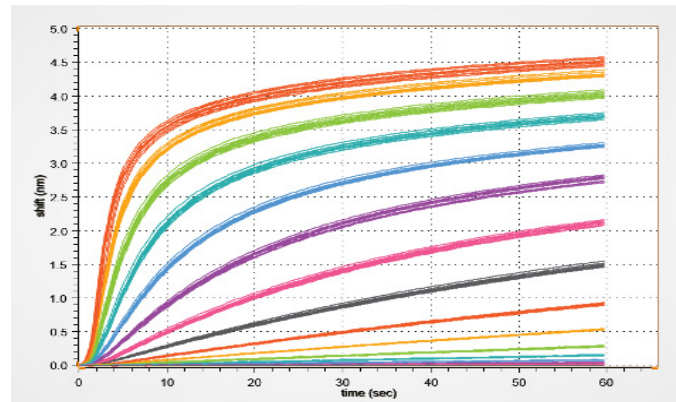


Figure 1: Quantitation of human IgG in Q buffer. IgG concentration ranges between 0.3 and 6000 µg/mL. The data was acquired in 60 sec at 400 rpm.

Conc (µg/mL)	Avg. Binding rate	% CV (Avg. Binding Rate)	Calculated Conc (µg/mL)	% CV (Calculated Conc.)
6000	1.36	2.8	6815	8.3
3000	1.00	2.4	3252	5.1
1500	0.6767	2.7	1575	4.6
750	0.4233	2.9	779.6	4.0
375	0.2485	2.1	389.8	2.6
188	0.1398	2.6	197.5	2.9
93.8	0.0722	2.3	94.7	2.5
46.9	0.0383	2.4	48.1	2.6
23.4	0.0199	1.5	24.4	1.5
11.7	0.0103	0.8	12.4	1.0
5.86	0.0051	2.0	6.10	2.0
2.93	0.0025	1.5	2.97	1.5
1.46	0.0012	2.0	1.50	1.8
0.73	0.0006	3.1	0.84	2.9
0.37	0.0003	8.2	0.44	7.1

Table 1: % CV of human IgG binding to HFCII and calculated concentration is <10 % over 20 regenerations.

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Kinetics

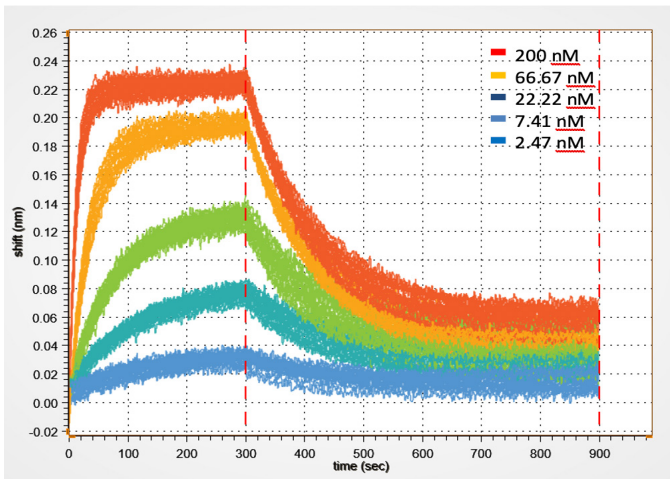


Figure 3: Association and dissociation of anti-RBD IgG1 (5 µg/mL) and RBD protein (2.47 - 200 nM; 1:3 dilution) measured at 1000 rpm over 20 regeneration cycles.

Cycles	k _{off} (1/s)	k _{on} (1/Ms)	K _D (M)
1	2.82E-03	3.70E+05	7.62E-09
2	2.84E-03	3.78E+05	7.50E-09
3	2.97E-03	3.87E+05	7.68E-09
4	2.65E-03	4.08E+05	6.49E-09
5	2.66E-03	4.08E+05	6.53E-09
6	3.20E-03	4.30E+05	7.44E-09
7	3.14E-03	4.05E+05	7.75E-09
8	3.31E-03	4.48E+05	7.38E-09
9	3.19E-03	4.39E+05	7.27E-09
10	2.85E-03	5.01E+05	5.69E-09
11	4.23E-03	4.26E+05	9.93E-09
12	3.92E-03	4.23E+05	9.26E-09
13	3.73E-03	4.46E+05	8.37E-09
14	3.34E-03	5.01E+05	6.67E-09
15	3.76E-03	4.62E+05	8.14E-09
16	4.33E-03	4.41E+05	9.80E-09
17	4.46E-03	4.46E+05	9.99E-09
18	3.71E-03	5.08E+05	7.29E-09
19	3.50E-03	5.43E+05	6.44E-09
20	3.91E-03	4.97E+05	7.86E-09

Table 2: Kinetics parameters for anti-RBD IgG1 and RBD protein over 20 regeneration cycles. k_{off}, k_{on} and K_D values are within 10x folds of each other.

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PN: 160024 - Gator® HFCII Probes

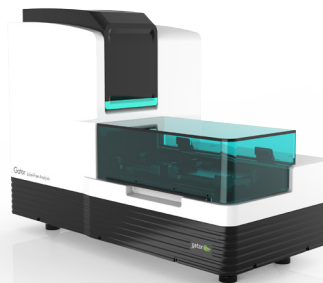
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