

Human Fc (HFC) Probes

Catalog No. 160003

OVERVIEW

Gator™ Human Fc (HFC) Probes are useful for measuring the concentration and kinetics of antibodies and proteins recognized by anti-human Fc. HFC probes utilize a high affinity anti-human Fc antibody and can capture the Fc region of human IgG1, IgG2, IgG3 and IgG4. Applications include quantitation of crude or purified samples, kinetics analysis of antibody-antigen to determine (k_{on} , k_{off} , K_D), off-rate screening, and isotyping of crude hybridoma cell lysate. These probes can be regenerated and reused for multiple experiments.

MATERIALS REQUIRED

Human Fc Probes	Catalog No. 160003
Max Plate	Catalog No. 130062
Black Plates	Greiner 655209
Quantitation (Q) Buffer	Catalog No. 120010
Kinetics (K) Buffer	Catalog No. 120011
Regeneration Buffer	Catalog No. 120012

STORAGE

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

GENERAL APPLICATIONS

1. Quantitation of crude or purified samples of immunoglobulins
2. Kinetics of interactions of an antigen with an antibody
3. Determination of concentration of an antibody and interaction with antigen in one run (QKR)
4. Epitope binning
5. Isotyping

GENERAL METHODS

Sample Volume

Black Plate: 200 μ L (180 μ L minimum)
 Max Plate: 250 μ L (280 μ L maximum)

Pre-wet Conditions

250 μ L in Max Plate, 5 min at 1000 rpm

Speed

Q	Standard Protocol: 400 rpm, 120 seconds; 5 μ g/mL – 300 μ g/mL
	High-Sensitivity Protocol: 1000 rpm, 300 seconds; 0.05 μ g/mL – 50 μ g/mL
K	1000 rpm
Q K R	Use 400 or 1000 rpm for the quantitation step (based on concentration) and 1000 rpm for the kinetics steps

Regeneration to Save Consumables

For kinetics applications, HFC probes can be regenerated over 20 times using the Gator™ software. (Settings are in Assay Setup.) Regeneration buffer and neutralization buffer (Q or K Buffer) should be placed in adjacent wells in either the black plate or the Max plate. For HFC probes, 3 cycles of 5 seconds for regeneration is recommended. A small loss in binding capacity is expected after each regeneration cycle, but this will not impact kinetics data. For the highest quality quantitation data, it is strongly recommended to use new probes for each sample. After regeneration, probes can be stored in assay buffer and kept at 4°C for >2 weeks.

Rapid Quantitation of Unknown Samples

Quantitation of crude or purified unknown samples can be performed using the Q assay preset on the software. For accurate results, make a standard curve of known concentrations in the same buffer as the unknowns. The linear range of HFC probes is 0.05 μ g/mL to 300 μ g/mL.

Known Concentration (μ g/mL)	Average Calculated Concentration (μ g/mL, n = 10)	Standard Deviation	% CV
300	309	24	7.7
150	144	3.8	2.6
75	77	1.2	1.5
37.5	37.7	0.36	0.9
18.8	18.6	0.3	1.6
9.38	9.31	0.23	2.5
4.69	4.74	0.11	2.4
2.34	2.34	0.06	2.6

Table 1: Quantitation of a standard curve of human IgG (in Q Buffer) 10 times using the same probes with regeneration in between.

Kinetics Application with Regeneration

HFC probes exhibit high affinity for antibodies, with an interaction stable enough for kinetics characterization of antigen interaction. Shown in Figure 1 is the association and dissociation of many concentrations of an antigen following loading of a human IgG. Additionally, HFC probes can be regenerated and used multiple times for kinetics applications with reproducible results (see Table 1).

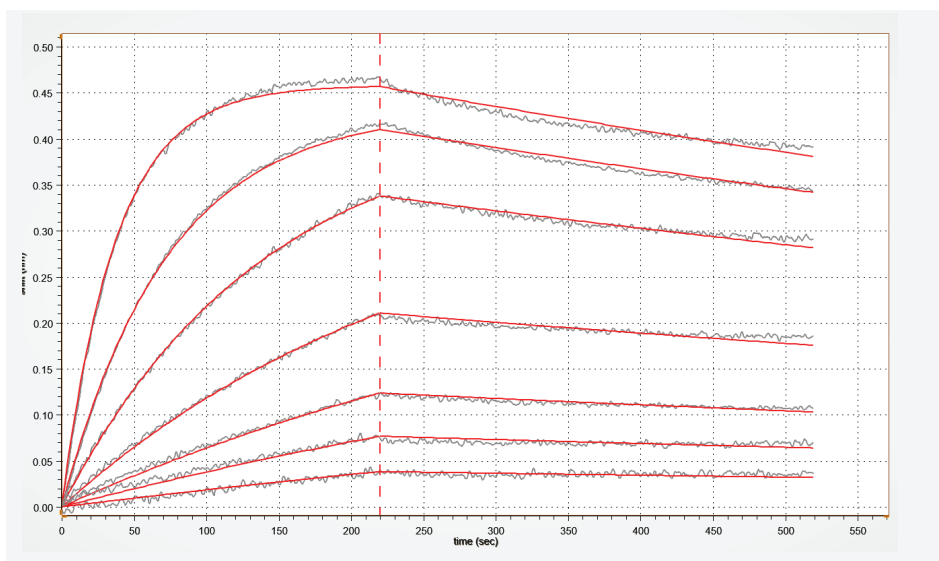


Figure 1: Human IgG (5 μ g/mL in K Buffer) was loaded onto HFC probes followed by association and dissociation of a range of concentrations (0.3 to 100 nM in K Buffer) of fluorescein-BSA. Global-fit analysis was performed (best-fit lines in red), and the affinity is 1.68 nM.

	k_{off} (1/s)	k_{on} (1/M*s)	K_D (nM)
Assay 1	6.60 e-4	3.92 e5	1.68
Assay 2	7.41 e-4	3.41 e5	2.17
Assay 3	6.21 e-4	3.46 e5	1.80
Assay 4	5.93 e-4	3.38 e5	1.76
Assay 5	4.87 e-4	3.27 e5	1.49
Assay 6	6.16 e-4	3.16 e5	1.95
Assay 7	5.80 e-4	3.04 e5	1.91
Assay 8	5.85 e-4	2.97 e5	1.97
Assay 9	6.82 e-4	3.03 e5	2.25
Assay 10	6.21 e-4	2.92 e5	2.12

Table 2: Human IgG (5 $\mu\text{g}/\text{mL}$ in K Buffer) was loaded onto HFC probes and the subsequent interaction with an antigen was measured. The experiment was repeated 10 times with the same probes using regeneration. The calculated affinity parameters are shown in the table.