

Gator® Anti-Human IgG Fc Gen II (HFCII) Probes
Part No. 160024
Overview

Gator® Anti-Human IgG Fc Gen II (HFCII) probes can detect, quantitate, and characterize the kinetics affinities of all human IgG antibodies (subclasses 1-4), recombinant human IgG antibodies and human IgG-derived Fc fused proteins. HFCII specifically binds to Fc region of human IgG antibodies and has similar binding affinities to all four subclasses of human IgG, without cross-reacting to IgGs of other species. HFCII is compatible with crude samples. Its ease and versatility make it useful in epitope binning, high-throughput applications, process development, isotyping of crude hybridoma, cell lysates, and hit-to-lead antibody discovery.

Application Summary

- Dynamic range: 0.3 - 6000 µg/mL at 400 rpm, 60 sec
- LoD: 0.1 µg/mL at 1000 rpm, 300 sec
- Precision/accuracy: < 10% CVs
- Regeneration: Up to 20 times in both Q and K assays
- Throughput: 8 samples in 2 min, 96 samples in 34 min

Materials Required

| | |
|------------------------|-----------------|
| Max Plate | Part No. 130062 |
| Gator BLI Plate | Part No. 130260 |
| Q Buffer | Part No.120010 |
| K Buffer | Part No. 120011 |
| Regen Buffer (No Salt) | Part No. 120063 |

Storage

Store HFCII probes (Part Number:160024) at room temperature in a foil pouch, ensuring zipper is fully sealed to avoid humidity/ moisture contamination. In high-humidity environments, storage inside a dry cabinet is recommended.

General Methods
Sample Volume

- Black Plate (96-well plate): 200 µL (180 µL minimum)
- Black Plate (384-well plate): 100 µL (80 µL minimum)
- Max Plate: 250 µL (280 µL maximum)

Pre-wet Conditions

- Use the same buffer or media as the samples being quantified are in to minimize background response due to nonspecific binding.
- 10 min at 1000 rpm when using buffers
- 30 min at 1000 rpm when using media

Regeneration

- Regenerate in Regen Buffer (No Salt) (PN: 120063) and neutralization buffer (buffer diluent) at 1000 rpm for 5 sec each, for a total of three cycles.
- For optimal performance, regenerate the probes once before starting the assay.
- The probes can be regenerated up to 20 times without showing significant loss of binding to human IgG Fc region.

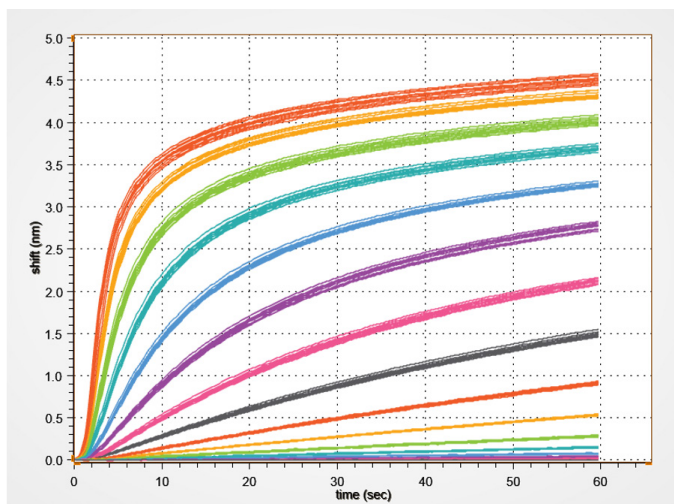
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Quantification Assay


Figure 1: Quantification of human IgG in Q buffer over 20 regeneration cycles. IgG concentration ranges between 0.3 - 6000 µg/mL. The data was acquired in 60 sec at 400 rpm.

Sample and Standard Preparation

- Quantify purified IgG and subtypes in Q buffer preferably.
- Prepare IgG standards in the same buffer or media that the samples are in. Use the same IgG isotype that is to be quantified in the samples.
- Dilute crude samples with Q buffer or pre-wet the probes in same media as the samples for at least 30 min and regenerate 4-5 times to stabilize the surface.
- Test several dilutions (1:2, 1:3, 1:4) of the samples to ensure that IgG concentration lies within the dynamic range of the probe.

Tips for Quantitation Assays

- Run a buffer diluent only as a reference for background subtraction during data analysis.
- Standard curves can be saved and used for subsequent experiments, if sample matrix is same.
- The concentration of sample(s) being analyzed should fall within the concentration range of the standard curve for an accurate quantification.

| Conc (µg/mL) | Avg. Binding Rate | % CV (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: Average binding rates of human IgG, calculated concentrations and their respective % CV after 20 regeneration cycles.

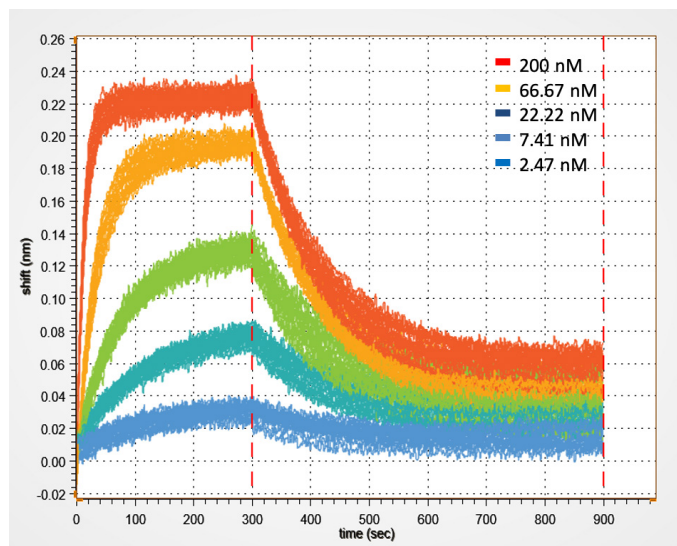
Kinetics Assay


Figure 2: Association and dissociation of anti-RBD IgG1 (5 µg/mL) and SARS-CoV-RBD protein (2.47 - 200 nM; 1:3 dilution) at 1000 rpm over 20 regeneration cycles. The association and dissociation curves for each run are globally fitted to 1:1 binding model.

Tips for Kinetics Assays

- For high analyte concentrations ($\geq 1 \mu\text{M}$), use Q buffer or add 1% BSA in Q buffer to avoid non-specific binding to the surface.
- Include a reference well that has no ligand for accurate baseline subtraction.
- Dedicate a separate reference well to check for non-specific binding of the ligand to the probes.
- Optimize the loading of human IgG or human derived-IgG Fc-fused protein to not exceed 1 nm signal shift. Slower loading speed (e.g., 400 rpm) can help improve immobilization.

Sample Preparation

- Perform kinetics assays in K buffer or Q buffer.
- Dilute ligand and antigen in same assay buffer.

| 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: k_{off} , k_{on} and K_D values for anti-RBD IgG1 and SARS-CoV-RBD protein interaction using HFCII probes over 20 regeneration cycles.

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PN: 160024 - Gator® HFCII Probes