PRODUCT INSERT

Protein A (Pro A) Probes

Catalog No. 160001

OVERVIEW

Gator™ Protein A (Pro A) Probes are useful in measuring the concentration and kinetics of antibodies. Specifically, Protein A binds to the heavy chain within the Fc region of most immunoglobulins, with a particularly high affinity for human IgG1, IgG2, and IgG4. In addition, Protein A has high affinity for mouse (IgG2 and IgG3) and rabbit immunoglobulins. These probes can be regenerated and reused for multiple experiments.

MATERIALS REQUIRED

Protein A Probes	Catalog No. 160001
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 assays of an antigen with an antibody
3. Determination of concentration of an antibody and interaction with antigen in one run (QKR)

GENERAL METHODS

Sample Volume

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

Pre-wet Conditions

250 µL assay buffer (Q or K) in Max Plate,
5 min at 1000 rpm

Speed

Q	**Standard Protocol:** 400 rpm, 120 seconds; 1µg/mL – 2000 µg/mL **High-Sensitivity Protocol:** 1000 rpm, 300 seconds; 25 ng/mL – 500 µg/mL
K	1000 rpm
QKR	Use 400 or 1000 rpm for the quantitation step (based on concentration) and 1000 rpm for the kinetics steps

Rapid Quantitation of Unknown Samples

Quantitation of crude or purified samples can be done using the Q assay preset on the Gator™ software. For accurate results, make a standard curve of known concentrations in the same buffer as the unknowns. The linear range of ProA probes is 25 ng/mL to 2 mg/mL. Probes can be regenerated in between samples to analyze an entire plate of samples in one run.

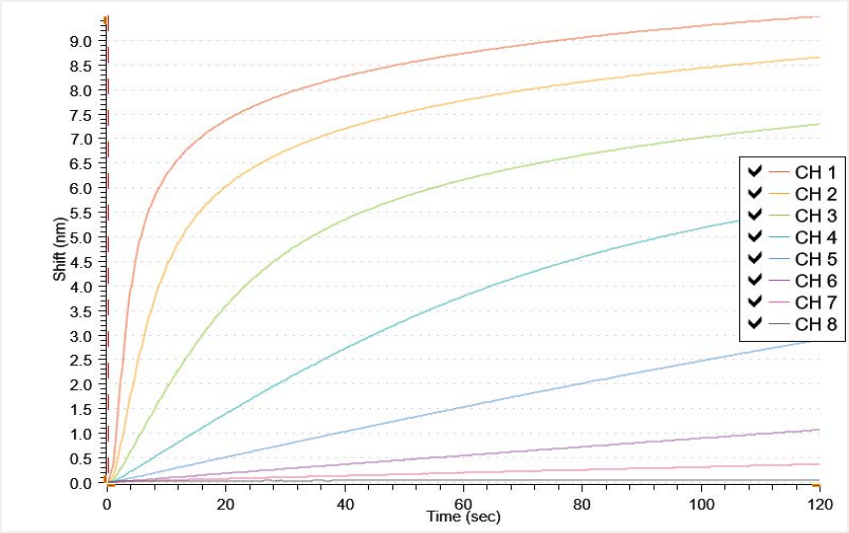

Figure 1: Binding curve of human IgG to Pro A probes (1 µg/mL to 900 µg/mL in Q Buffer). Assay performed using standard protocol (400 rpm for 120 sec).

Regeneration

Pro A probes can be regenerated using the Gator™ software. (Settings are in Assay Setup.) Regeneration buffer (PN 120012) and neutralization buffer (Q or K Buffer) should be placed in adjacent wells in either the Black Plate or the Max Plate. 3 cycles of 5 seconds for regeneration is recommended. Regeneration before assay is recommended to ensure run-to-run consistency. After regeneration, probes can be stored in assay buffer and kept at 4°C for >2 weeks.

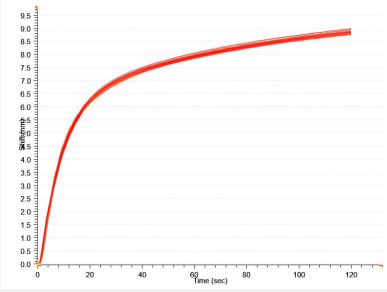

Figure 2: 10 consecutive measurements of human IgG (900 µg/mL in Q Buffer) on the same Pro A probe with regeneration. Assay performed using standard protocol (400 rpm for 120 sec).

Important Note: We recommend rerunning the standard curves with every new lot of Pro A probes.

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