

Protein G (Pro G) Probes

Catalog No. 160006

OVERVIEW

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

MATERIALS REQUIRED

Protein G Probes	Catalog No. 160006
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 antibodies
3. Determination of concentration of an antibody and interaction with antigen in one run (QKR)

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

Speed

Q	Standard Protocol: 400 rpm, 120 seconds; 1 μ g/mL – 2000 μ g/mL
	High-Sensitivity Protocol: 1000 rpm, 300 seconds; 25 ng/mL – 500 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

Rapid Quantitation of Unknown Samples

Quantitation of crude and purified unknown samples can be performed 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 Pro G 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.

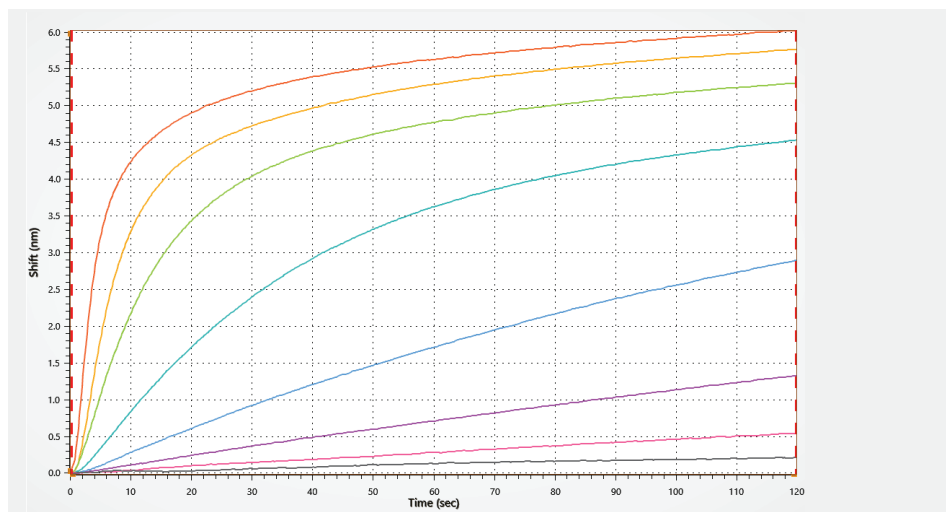


Figure 1: Binding curve of mouse IgG to Pro G probes (1 μ g/mL to 2000 μ g/mL in Q Buffer). Assay performed using standard protocol (400 rpm for 120 sec).

Regeneration to Save on Consumables

Pro G probes can be regenerated 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 Pro G probes, 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.

Kinetics Assay with Regeneration

Pro G probes can be used to measure antibody-antigen interaction (Figure 2). This application can be used for off-rate ranking and affinity measurement. The probes can be regenerated for kinetics application with reproducible results (Table 1).

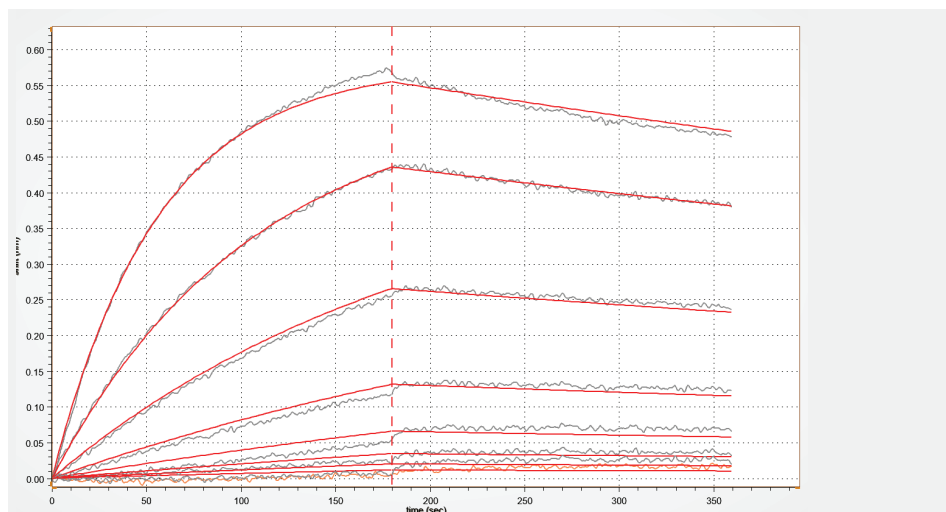


Figure 2: A kinetics assay was performed with ProG probes by loading mouse anti-fluorescein (5 μ g/mL in K Buffer; 1000 rpm) followed by association and dissociation of a range of concentrations of fluorescein-BSA (30 nM to 0.3 nM in K Buffer; 1000 rpm). Shown is the kinetics global-fit with best-fit lines in red (K_D is 1.93 nM, $r^2 = 0.98$).

	k_{off} (1/s)	k_{on} (1/M*s)	K_{D} (nM)
Assay 1	9.94 e-4	5.14 e5	1.93
Assay 2	8.73 e-4	5.15 e5	1.70
Assay 3	7.90 e-4	4.95 e5	1.60
Assay 4	7.41 e-4	4.74 e5	1.57
Assay 5	7.90 e-4	4.58 e5	1.72
Assay 6	7.85 e-4	4.50 e5	1.74
Assay 7	7.51 e-4	4.45 e5	1.69
Assay 8	7.42 e-4	4.53 e5	1.64
Assay 9	7.35 e-4	4.32 e5	1.70
Assay 10	7.24 e-4	4.20 e5	1.73

Table 1: The calculated k_{off} , k_{on} , and K_{D} values of mouse anti-fluorescein and fluorescein-BSA binding measured using Pro G probes with regeneration in between assays.