

Mouse Fc (MFC) Probes

Catalog No. 160004

OVERVIEW

Gator™ Mouse Fc (MFC) Probes are useful for measuring the concentrations and kinetics of antibodies and mouse-Fc fusion proteins. MFC probes have a high-affinity anti-mouse Fc antibody immobilized on the surface. The Fc region of mouse IgG1, IgG2a, IgG2b can be captured on this surface with a high affinity stable interaction. (Mouse IgG3 should be evaluated on a case-by-case basis). Applications include quantitation of mouse and rat IgGs, kinetics analysis of antibody-antigen to determine (k_{on} , k_{off} , K_D), off-rate screening, and isotyping of crude hybridoma cell lysates. These probes can be regenerated and reused in multiple experiments.

MATERIALS REQUIRED

| | |
|-------------------------|--------------------|
| Mouse Fc Probes | Catalog No. 160004 |
| Max Plate | Catalog No. 130062 |
| Black Plates | Greiner 655209 |
| Quantitation (Q) Buffer | Catalog No. 120010 |
| Kinetics (K) Buffer | Catalog No. 120011 |
| MFC Regeneration Buffer | Catalog No. 120008 |

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

Speed

| | |
|-------------|--|
| Q | Standard Protocol: 400 rpm, 120 seconds; 5 μ g/mL – 100 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 |

Rapid Quantitation of Unknown Samples

Quantitation of crude or 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 MFC probes is 0.05 μ g/mL to 100 μ g/mL.

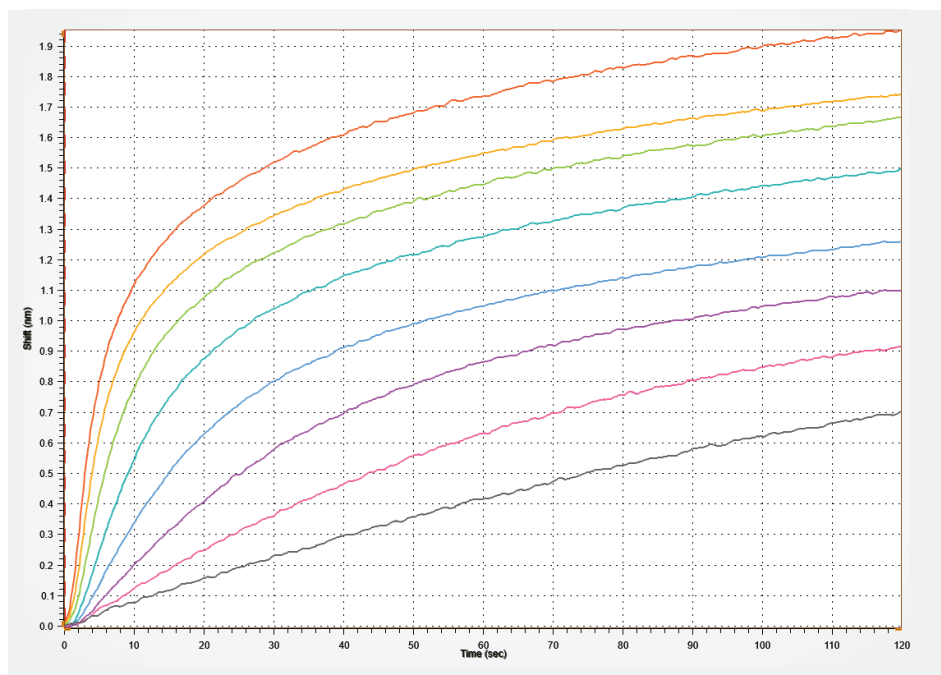


Figure 1: Binding curve of mouse IgG to MFC Probes (0.03 to 100 μ g/mL in Q Buffer). Assay performed using standard protocol (400 rpm, 120 sec).

Regeneration to Save Consumables

For kinetics applications, MFC 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 MFC 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.

Tips for Optimal Performance

For the best performance, it is recommended to regenerate the probes using MFC Regeneration Buffer (Cat No. 120008) prior to use.