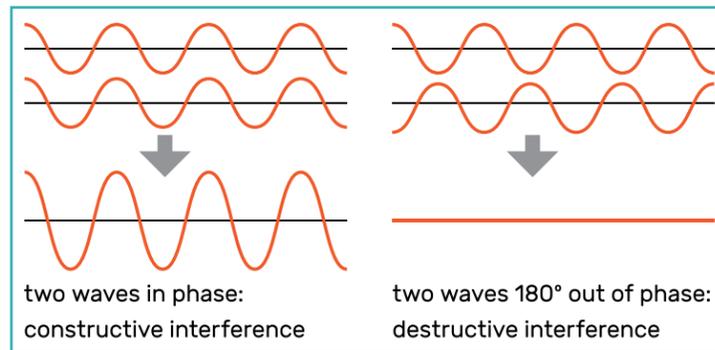


Gator Bio Applications Book



Bilayer Interferometry (BLI)

In bilayer interferometry, biomolecular interactions are detected by measuring the interference pattern of white light reflected from the surface of a biosensor.



Gator™ biosensors combine a 1mm diameter glass rod with patented optical layers and specialized surface chemistry built at the distal end of the biosensor.

Bilayer interferometry compares the interference pattern of white light reflected from an internal reference layer within a layer of immobilized biomolecules on the surface chemistry of the biosensor.

Shifts in interference due to the accumulation of biomolecules are monitored in real-time to sensitively analyze and calculate rates of association and dissociation among target proteins with high precision.

Only the interactions of bound biomolecules are measured, making bilayer interferometry a powerful tool for a wide range of applications.

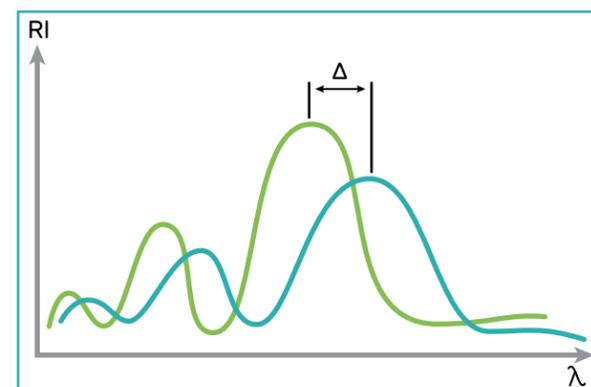
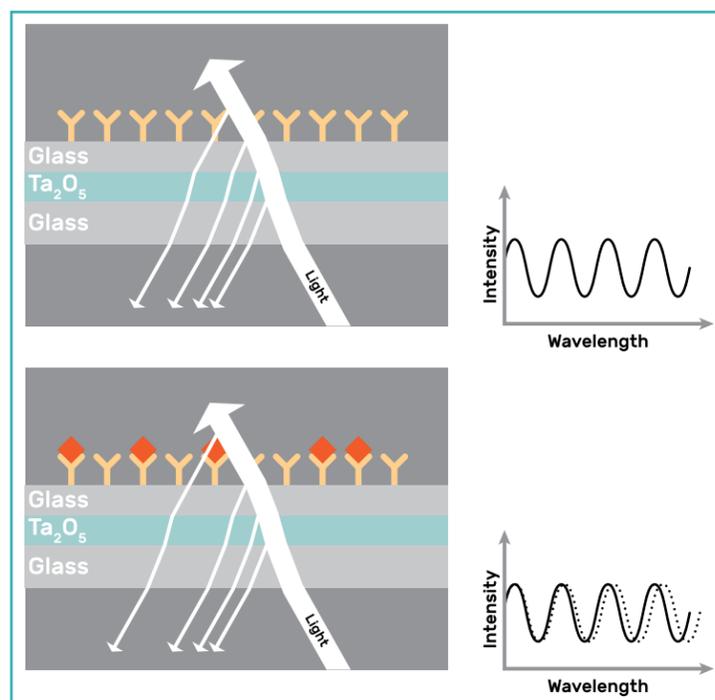
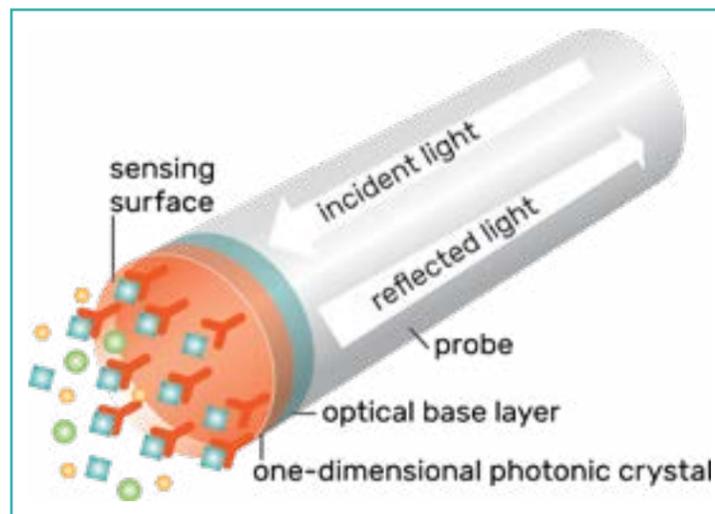


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Gator™ AAVX Probes

Rapid and Label-free Quantitation of AAV Serotypes

Introduction

Adeno-associated virus (AAV) capsids are a leading modality for in vivo gene delivery. Complete and precise characterization of capsid particles, including capsid and vector genome concentration, is necessary to safely and efficaciously dose patients. In virus development and production, it is important to determine the virus concentration at different stages of the process, to optimize the clone used as well as the production yields. Biolayer Interferometry (BLI) using AAV specific biosensors is a rapid label-free method for quantitation of AAV serotypes.

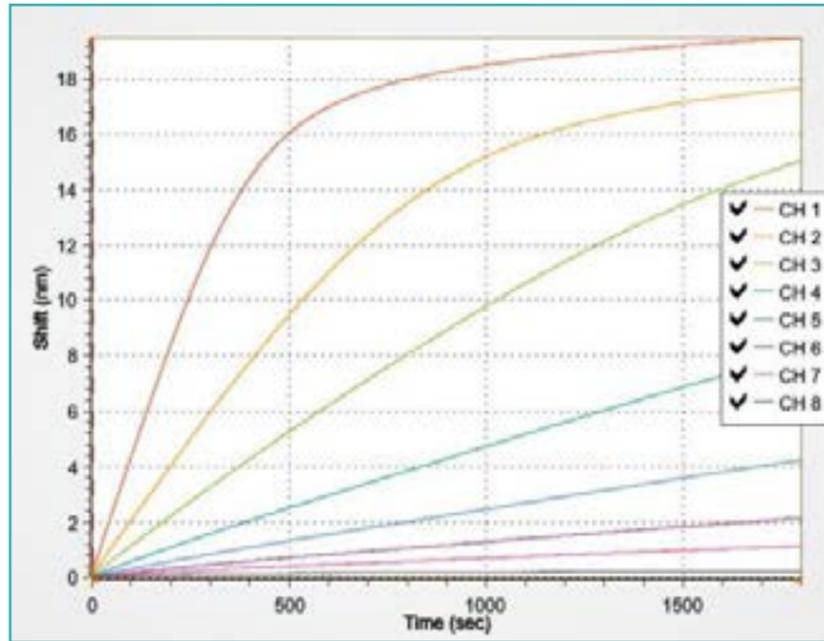
Gator™ AAVX Probes

The Gator™ AAVX probes are high specificity antibody-based biosensors that enable direct capture and quantitation of different serotypes of AAV in crude lysates, column eluates, cell lysates and cell culture supernatants, serving as an alternative to traditional time-consuming analytical methods, such as qPCR, ddPCR, Dot blot and ELISA. The Gator™ AAVX probe uses proven CaptureSelect™ (Thermo Fisher Scientific) high affinity and high specificity anti-AAVX antibody.

Performance Summary

- Dynamic range: 1×10^9 – 1×10^{13} vp/mL for most AAV serotypes
- Throughput: 8 samples in 10 minutes, 96 samples in 120 minutes
- Limit of detection: typically, 5×10^8 vp/mL (serotype dependent)
- Crude sample tolerant
- Stable over broad pH range
- Cost effective–Reusable at least 10 times by regeneration





Results

Dynamic range:

The AAV2 serotype range was tested using the Gator™ AAVX probes. The data shows 4 orders of magnitude dynamic range. The data was acquired using 1:3 dilution in 10 min at 1,000rpm.

Figure 1

Capture of AAV2 serotype on the standard curve

Table 1

Example of AAV8 serotype anti-AAVx

Channel Number	Known Conc. (vp/mL)	Binding Rate	Calc. Conc. (vp/mL)
1	3.33×10^{11}	0.069	3.41×10^{11}
2	1.67×10^{11}	0.0304	1.69×10^{11}
3	8.33×10^{10}	0.0119	7.62×10^{10}
4	4.17×10^{10}	0.00779	5.31×10^{10}
5	2.08×10^{10}	0.0023	1.87×10^{10}
6	1.04×10^{10}	0.00115	1.02×10^{10}
7	5.21×10^9	0.000592	5.55×10^9
8	0	0	0

Table 2

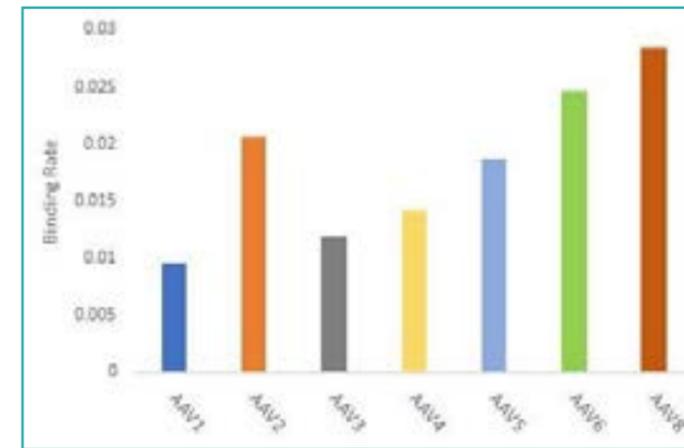
Accuracy and reproducibility

AAV5 serotype was used to test the accuracy and reproducibility. The table below shows recovery close to 100% and CV ranging from around 1.5% at medium and high titers to 10.9% at low titer.

Titer Level	Known Conc. (vp/mL)	Average Binding Rate	Average Calculated concentration (vp/mL)	% Recovery	% CV (n = 3)
High	2.00×10^{12}	0.11069	2.03×10^{12}	102	1.50
Medium	5.10×10^{10}	0.0045	5.27×10^{10}	103	1.81
Low	8.30×10^8	0.00010	8.30×10^8	100	10.9

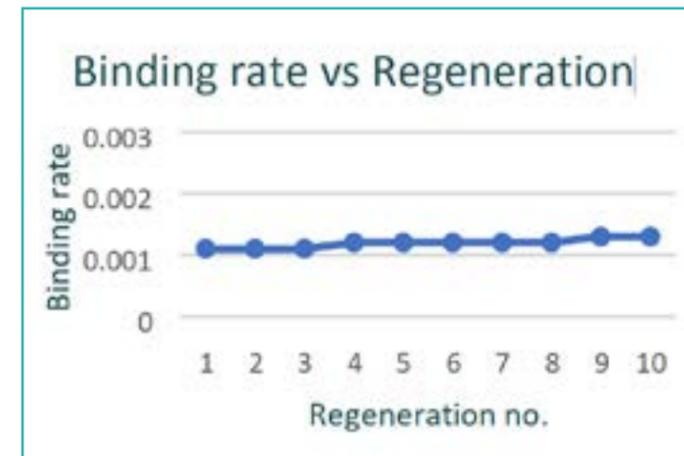
Binding rate of different AAV serotypes

The binding rate of 7 different serotypes of AAV were studied. The figure below shows binding rates of tested serotypes at 2×10^{11} vp/mL concentration.



Regeneration performance

Data below shows performance after 10 regenerations of the same probe. No loss in binding rate observed even after 10 regenerations.



Ordering Information

PL168-160017: Gator™ AAVX Probes

Gator™ SMAP Probes

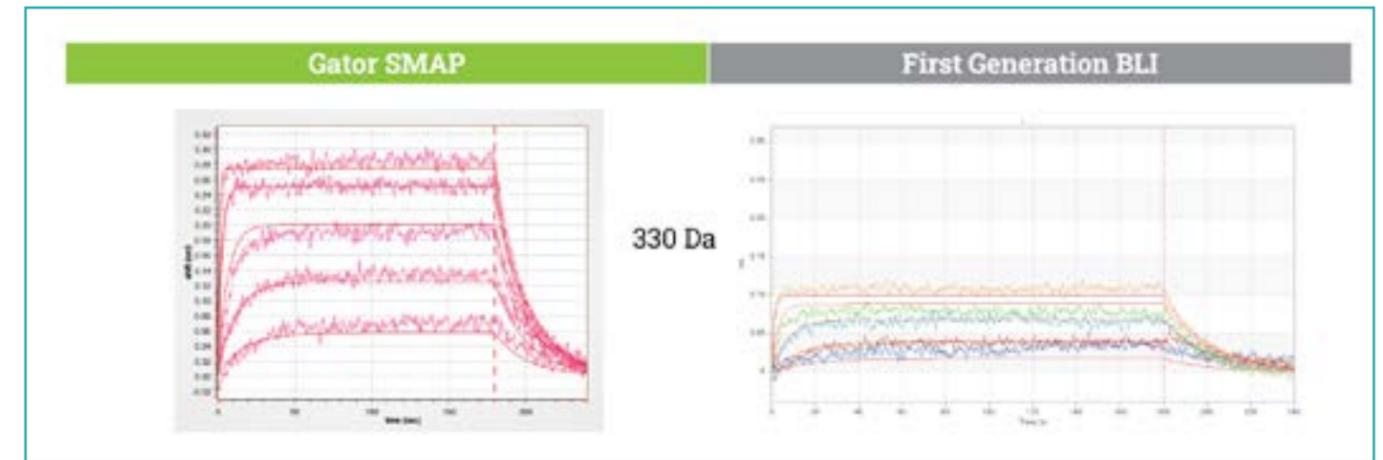
Small Molecule Sensitivity: The Power of Next Generation BLI

Overview

Gator Bio has recently launched the SMAP (Small Molecule, Antibody, and Protein) biosensor. As with other Gator™ biosensors, SMAP has fundamental physical and chemical design elements that provide much higher sensitivity. This biosensor is sensitive enough to capture small molecules (down to 150 Da), but can also be used for biomolecules and antibodies when sensitivity is a must. Compatible with our GatorPrime™ and GatorPlus™ instruments, SMAP biosensors bring a whole new level of sensitivity to biolayer interferometry (BLI) technology. Gator Bio is committed to innovation and improving upon ordinary BLI to help users.

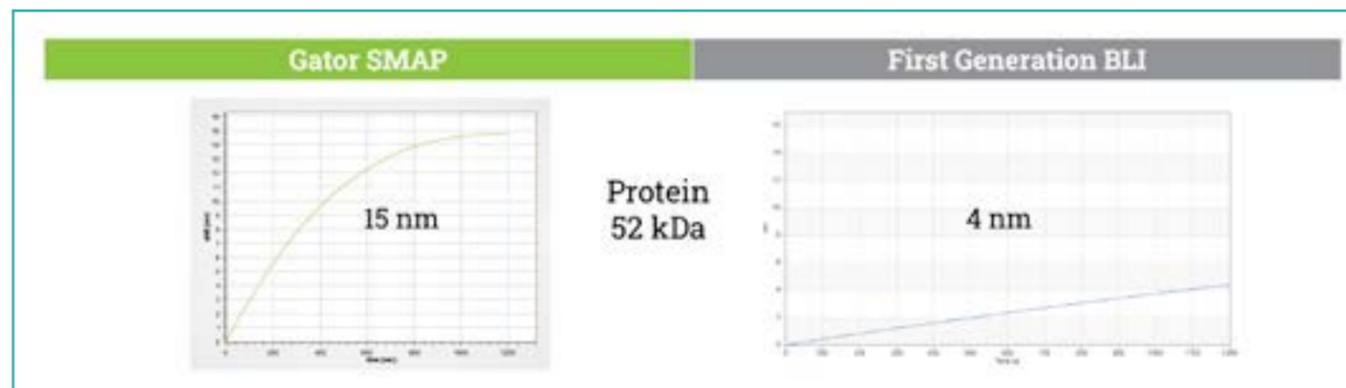
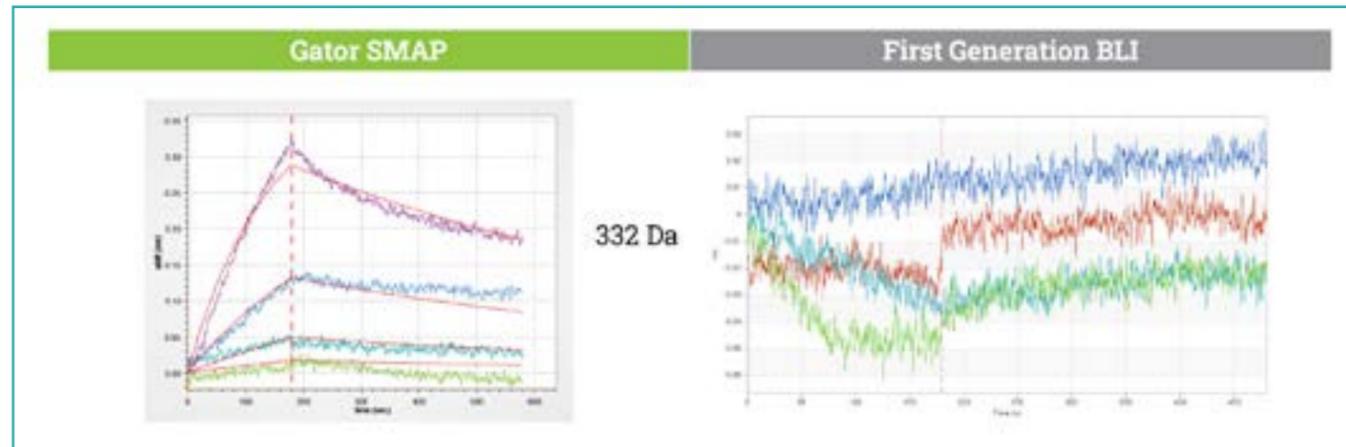
Benefits of SMAP

- Higher sensitivity
- More signal
- Compatible with small molecules and biomolecules of a wide range of sizes



Ordering Information

Catalog No. 160011: Gator™ SMAP Probes



Gator™ Anti-Mouse Fc Probes

Rapid Hybridoma Screening with Next-Gen BLI Based Highly Sensitive Anti-Mouse Fc Biosensor

Bi-layer interferometry (BLI) has been widely accepted for antibody screening, but one of the most widely used biosensors in mouse IgG quantitation, anti-mouse Fc, has always remained a step behind in performance standards. As the therapeutic antibody sector grows, the capacity and cost-per-assay demands will continue to exceed what traditional methods can produce. Gator Bio's next generation BLI systems with novel Gator™ Anti-Mouse Fc biosensors surpass traditional BLI's limitations with an expanded dynamic range for faster antibody screening. This next gen BLI based high sensitivity assay improves workflow while producing fast results with significant cost savings.

Streamlined workflow

Next-gen BLI with Anti-Mouse Fc biosensors can detect antibody concentrations from 0.02–2,000 µg/mL. This wide dynamic range enables researchers to rapidly screen hybridoma samples without the dilution steps necessary for traditional BLI and ELISA. This workflow improvement makes sample preparation fast and easy. There is no compromise of data quality across the broad dynamic range.

Known concentration (µg/mL)	Avg. calculated concentration (µg/mL)	CV (%)
2000	1967	4.9
667	690	1.0
222	215	1.3
74.1	75.1	1.0
24.7	24.7	3.2
8.2	8.2	2.2
2.7	2.7	3.6

Table 1:

Calculated average concentrations and CV% ranging from 2.74–2000 µg/mL

Quantitation of low antibody expression

Detection of antigen-specific IgG's in the low nanogram per mL range allows for quantification of low antibody expression of mouse hybridoma samples previously undetected by traditional BLI.

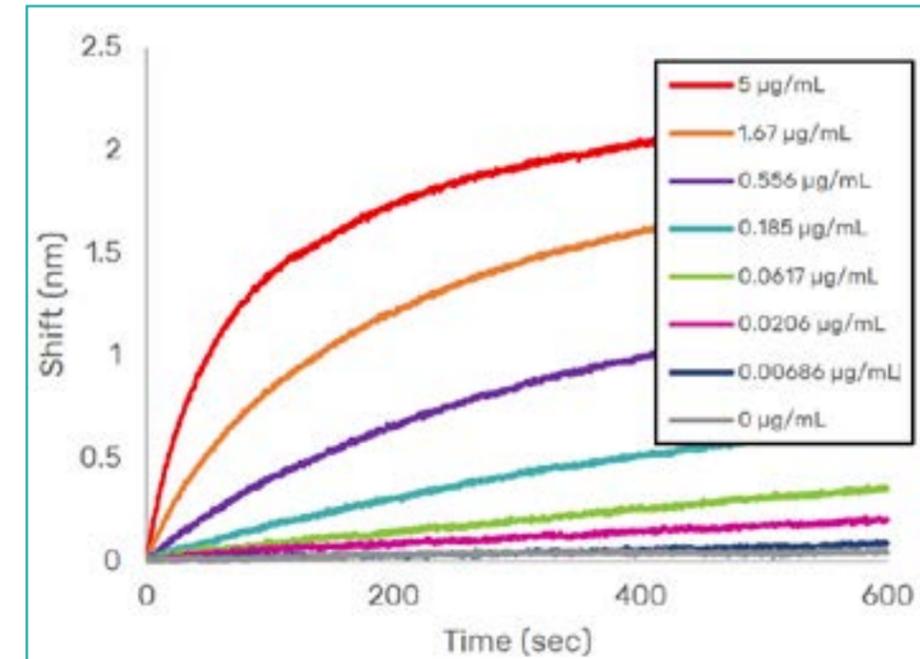


Figure 1:

Detected concentrations in mouse hybridoma samples ranging from 0–5 µg/mLⁱ

ⁱ Experiments in Figure 1 performed with GatorPlus™

Fast assays for quick time to results

The high sensitivity of the Gator™ Anti-Mouse Fc biosensor detects binding of antibodies within 30 seconds. Fast antibody detection allows for faster assay time and significant time savings.

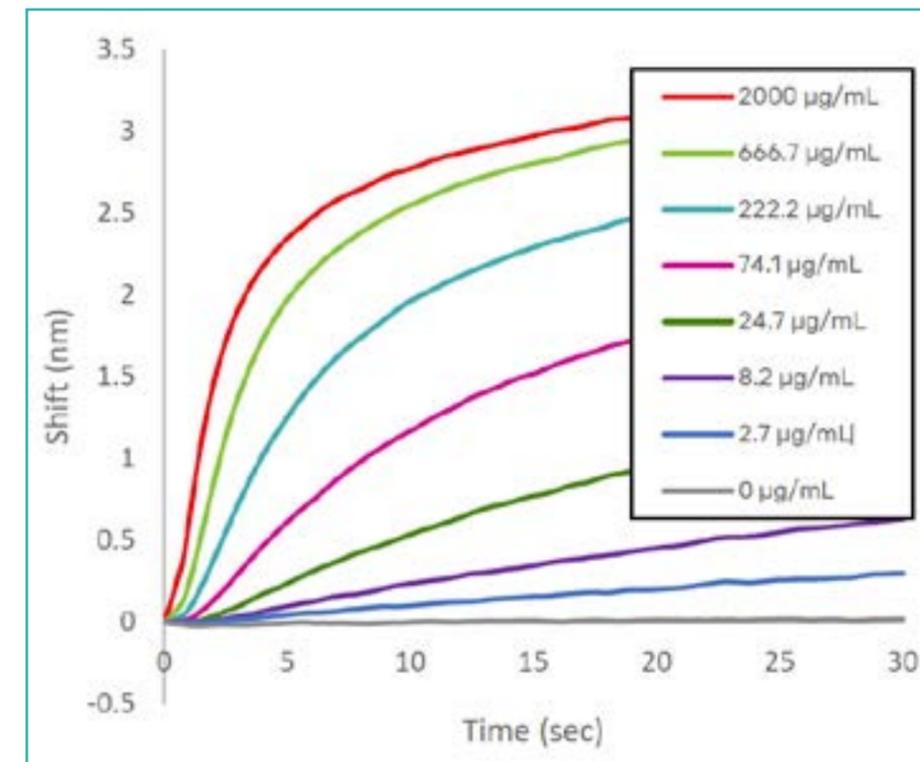


Figure 2:

Detected concentrations in mouse hybridoma samples ranging from 0–2000 µg/mL

Performance at a Glance

	Gator Bio BLI	Traditional BLI
Dynamic Range	0.02–2000 (µg/mL)	1–100 (µg/mL)
Throughput	8 samples/30 sec.	8 samples/120 sec.
Regeneration	20x	Not capable
Limit of quantitation	0.02 µg/mL	1 µg/mL

The Next Generation of Biolayer Interferometry

GatorPrime™ and GatorPlus™ next generation BLI systems provide fast and cost-effective solutions to achieve performance standards beyond the capabilities of traditional BLI. The novel polymer surface chemistry of the Gator™ Anti-Mouse Fc biosensors supports 20 or more rounds of regeneration to reduce cost-per-assay without compromising quality. In addition, the same biosensor can be used for both quantitation and kinetics in one experiment, saving on hands-on time.

Ordering Information

Catalog No. 160004: Gator™ Anti-Mouse Fc Probes

Gator™ Ni-NTA Kit

Quantitation and Kinetic Analysis of His-tagged Proteins

Introduction

Addition of a polyhistidine tag (His-Tag) to the N- or C-terminus of proteins is commonly used for immobilized metal ion affinity chromatography (IMAC) purification of target proteins. The Gator™ Ni-NTA probes allow for rapid and continuous quantification of His-tagged proteins without the need for Ni²⁺ recharging. The stable immobilization of His-tagged proteins allows for facile kinetic analyses with binding partners, off-rate screening, and epitope binning of antibodies experiments.

Gator™ Ni-NTA Probe

Gator™ Ni-NTA probes are functionalized with Qiagen™ Tris-NTA and charged with Ni²⁺ ions for high affinity immobilization of His-tagged proteins. The probes can be used for quantitation of His-tagged proteins in sample buffer and cell culture supernatants. The specially formulated regeneration and neutralization buffers allow for continuous regeneration of the probes, without the need for an additional Ni²⁺ recharging step.

Performance Summary

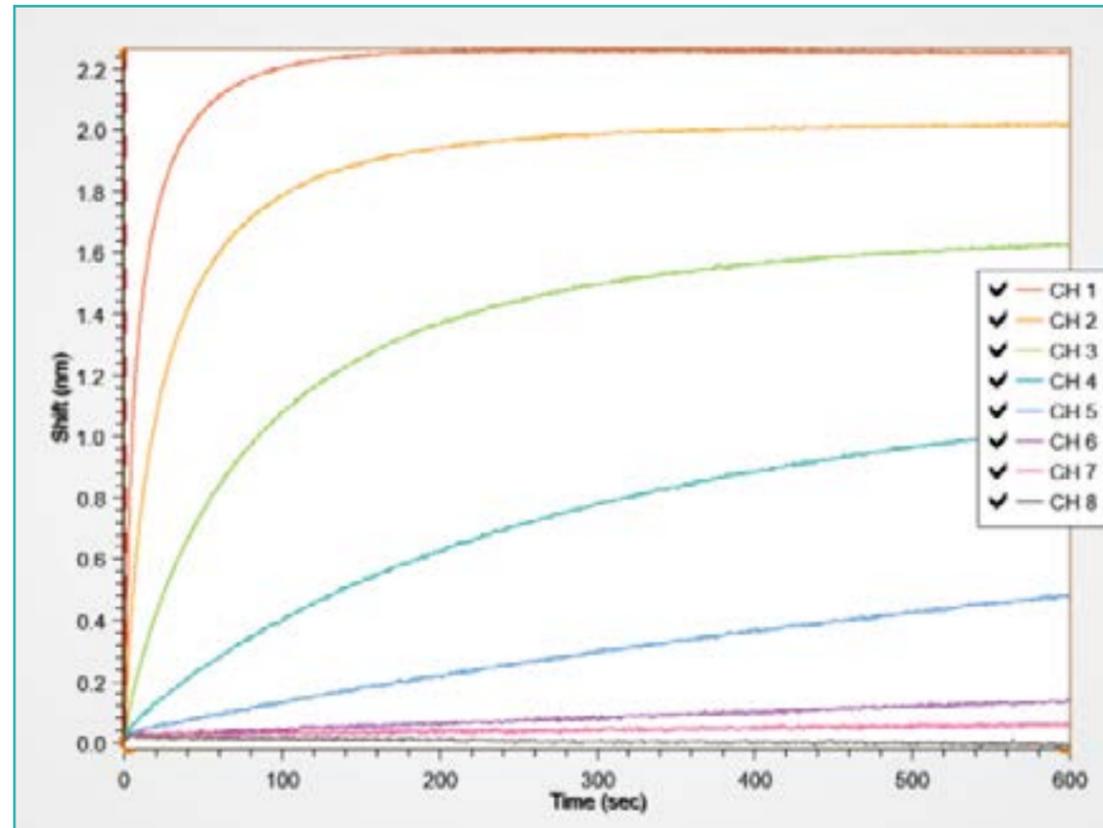
- Dynamic range: 0.25–1000 µg/mL (in Q buffer); 1–1000 µg/mL (in diluted cell culture media)
- Throughput: 8 samples in 2 minutes, 96 samples in 34 minutes
- Limit of detection: 0.25 µg/mL (10 min, 1500 rpm)
- Crude sample tolerant
- Cost effective: Reusable at least 20 times by regeneration in Q buffer; 10 times in diluted cell culture media



Results

Dynamic range

The dynamic range of Gator™ Ni-NTA probes was tested using His-tagged Protein A diluted in Q buffer. The data was acquired in 10 min at 1,500rpm.

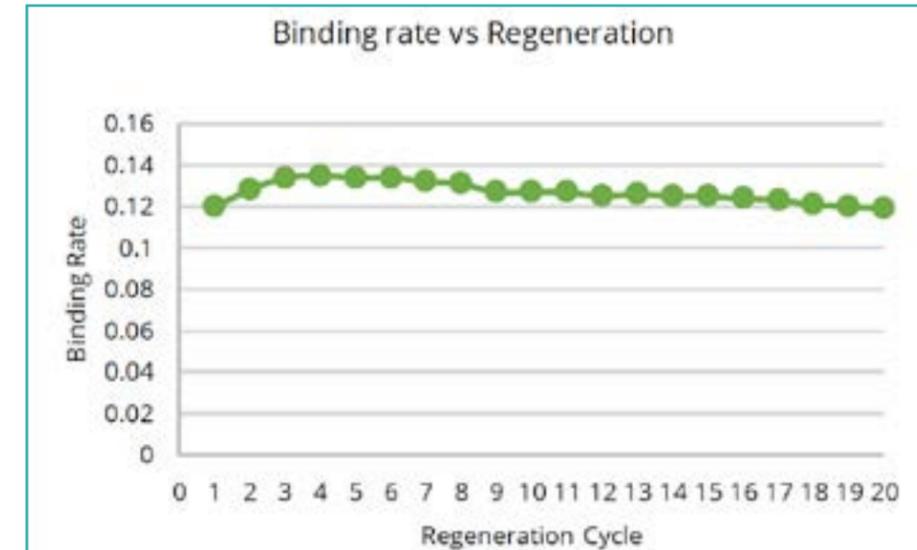


Known Conc. (µg/mL)	Calculated Conc. (µg/mL)	Binding Rate
1000	1018.307	0.3109
250	250.37	0.1038
62.5	65.731	0.0266
15.625	15.457	0.0051
3.906	4.05	0.0011
0.977	1.081	0.0003
0.244	0.25	0.0001

Regeneration performance

Quantitation

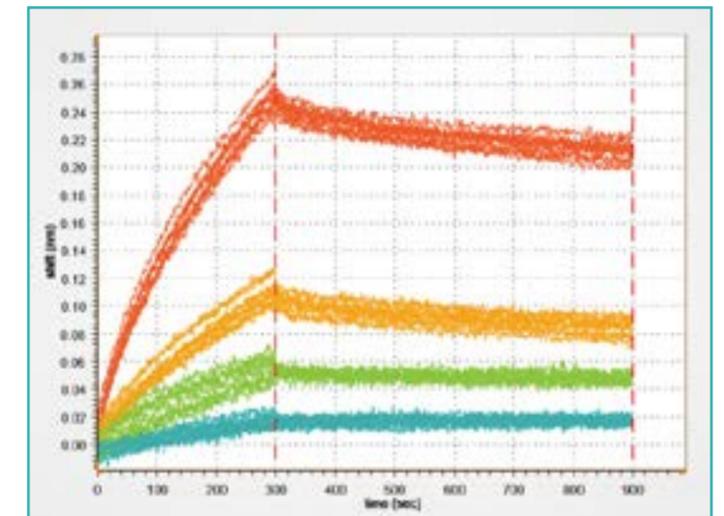
Figure below shows the quantitation performance after 20 regenerations of the same probe. No loss in binding rate observed even after 20 regenerations.



Kinetics

Similar kinetics parameters (k_{on} , k_{off} , K_D) of the binding between human IgG with CD64 were obtained following 10 regeneration cycles.

	k_{off} (1/s)	k_{on} (1/Ms)	K_D (M)
1	2.54×10^{-4}	1.39×10^4	1.83×10^{-8}
2	3.34×10^{-4}	2.25×10^4	1.48×10^{-8}
3	3.25×10^{-4}	1.39×10^4	2.34×10^{-8}
4	2.78×10^{-4}	2.00×10^4	1.39×10^{-8}
5	3.05×10^{-4}	1.78×10^4	1.71×10^{-8}
6	2.72×10^{-4}	1.66×10^4	1.64×10^{-8}
7	2.58×10^{-4}	1.59×10^4	1.62×10^{-8}
8	2.68×10^{-4}	1.48×10^4	1.81×10^{-8}
9	2.90×10^{-4}	1.78×10^4	1.63×10^{-8}
10	3.04×10^{-4}	2.17×10^4	1.40×10^{-8}



Ordering info:

PL168-350002: Gator™ Ni-NTA Kit

Gator™ Flex SA Kit

A Reactivable Streptavidin Probe Kit

Introduction

Many of the label-free biosensors have been regenerated successfully to reduce cost for drug research and discovery. However, extensive application of streptavidin biosensors is still hindered by the irreversible immobilization of the bait molecule, and the ability to use the same probe for different applications is desirable for cost-effective research.

Gator™ Flex SA Kit

Gator Bio's Gator™ Flex SA Kit is the first in the market to provide reactivable streptavidin probes with a reagent set. One probe can easily be used by different users with different applications, which makes it very useful for academics or for use in fully automated screening of different biomolecular interactions. The Gator™ platform enables researchers to monitor the whole reactivation process rapidly, and provides flexibility and confidence.

Performance Summary

- Remove both capture reagent and biotin-bait molecule and immobilize new capture reagent < 5 minutes
- Use with same or different biotinylated bait
- Can be re-used 10 times or more without loss of performance
- Automated reactivation
- Reagents can be stored at 4°C for up to 5 months



Results

Loading height reproducibility

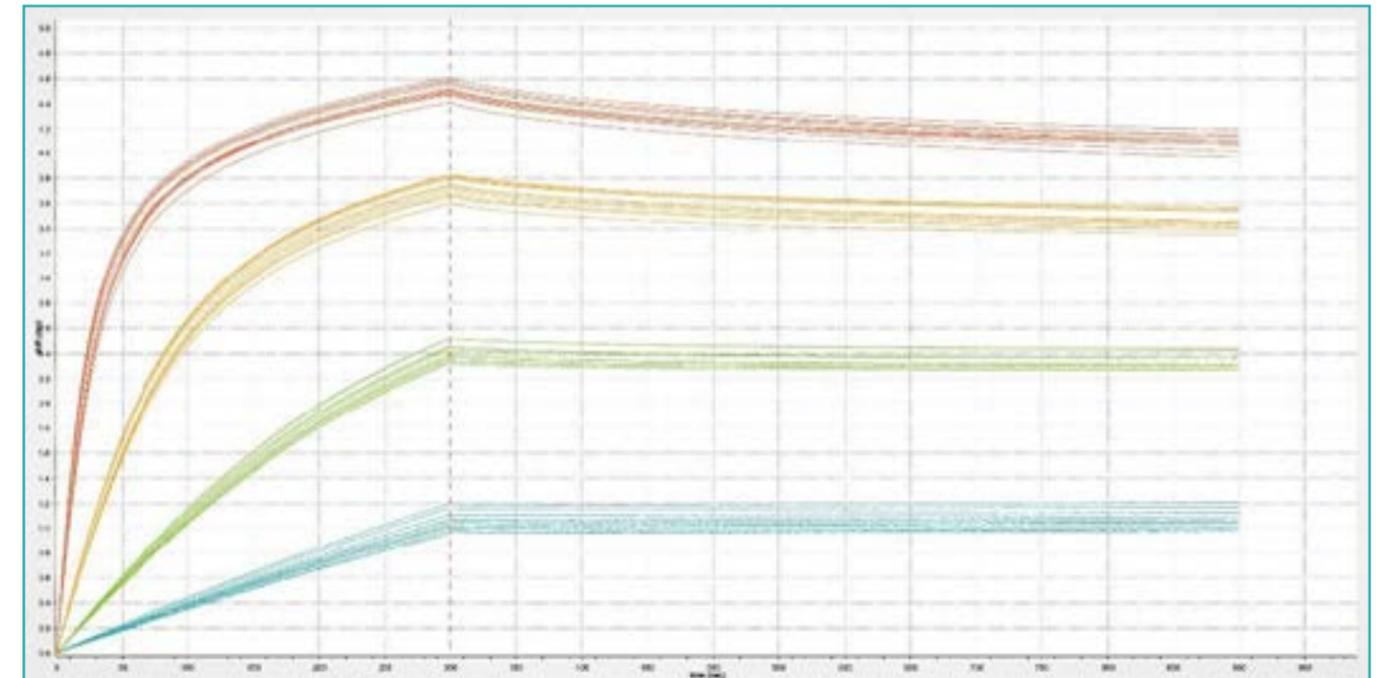
Three Flex SA probes were tested over 10 reactivations using biotinylated TNF-α at 1 μg/mL. The table below shows CV < 5.0% suggesting excellent loading height reproducibility.

Biotinylated TNF-α Loading Height

	Probe 1 (n=10)	Probe 2 (n=10)	Probe 3 (n=10)
Loading height avg (nm)	0.66	0.67	0.62
Loading height SD	0.03	0.03	0.03
Loading height % CV	4.54	4.09	5.4

Reproducibility of K_D over 10 reactivations using the same bait

Kinetics was evaluated using biotinylated TNFα and anti-TNFα antibody using a 5 min 1000 rpm pre-wet in K buffer and capture of Flex SA Capture Reagent (SA-CR). The biotinylated TNF-α was loaded onto the sensor, then exposed to association and dissociation of 10, 30, 100 and 300 nM anti TNF-α antibody. Global-fit analysis using Gator™ GatorOne Software for the TNF-α binding interaction with anti-TNF-α resulted in K_D = 7.63 x 10⁻¹⁰ M.



K_D measurements for different proteins over 10 reactivations

The same probe was tested with 2 different proteins over 10 reactivations. A CV < 5% was observed for both proteins.

	Bait 1—PDL1		Bait 2—CRP
Reactivation 1	2.00 x 10 ⁻¹⁰	Reactivation 2	4.77 x 10 ⁻¹⁰
Reactivation 3	1.08 x 10 ⁻¹⁰	Reactivation 4	4.28 x 10 ⁻¹⁰
Reactivation 5	1.17 x 10 ⁻¹⁰	Reactivation 6	3.88 x 10 ⁻¹⁰
Reactivation 7	1.06 x 10 ⁻¹⁰	Reactivation 8	3.49 x 10 ⁻¹⁰
Reactivation 9	0.40 x 10 ⁻¹⁰	Reactivation 10	3.22 x 10 ⁻¹⁰
Average	1.14 x 10 ⁻¹⁰	Average	3.93 x 10 ⁻¹⁰

Ordering Information

PL168-350001: Gator™ Flex SA Kit

Gator™ Streptavidin (SA) Probes

Overview

Gator™ Streptavidin (SA) Probes are useful for the study of biotinylated proteins. The proprietary surface chemistry allows for capture of both biotinylated proteins (with a recommended molar coupling ratio of less than three) or proteins expressed with an AviTag™. Following capture, the ability of the biotinylated protein of interest to bind to secondary proteins can be measured to determine the k_{on} , k_{off} , and K_D of interaction.

General Applications

1. Kinetics studies of protein-protein interaction
2. Indirect quantitation assays

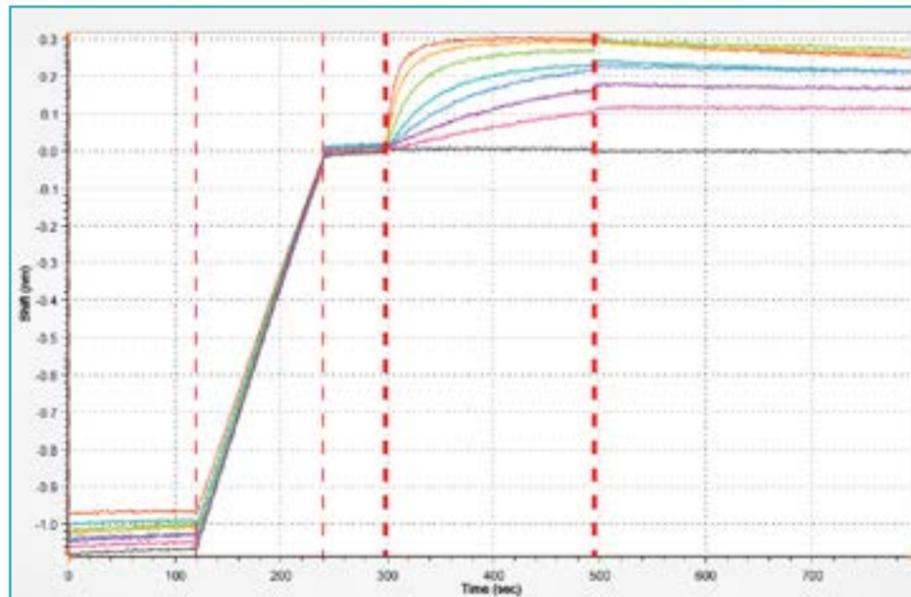


Figure 1:
Affinity measurement for protein-protein interactions. Following a baseline measurement in K Buffer, biotinylated rabbit IgG (1 µg/mL in K Buffer) was loaded onto SA Probes (400 rpm; 120 sec) followed by association and dissociation of an antigen over a range of concentrations (0–500 nM in K Buffer).

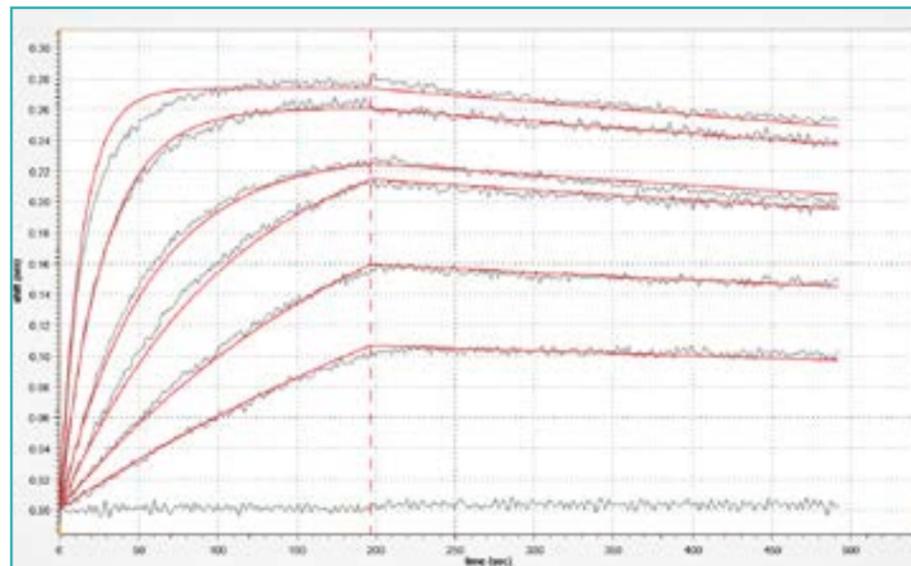


Figure 2:
Global-fit analysis using Gator™ GatorOne Software for antibody-antigen interaction shown in Figure 1. $K_D = 2.27$ nM ($R^2 = 0.99$)

Ordering Information

Catalog No. 160002: Gator™ Streptavidin (SA) Probes

Gator™ Protein A (Pro A) Probes

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.

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)

Rapid Quantitation of Unknown Samples

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

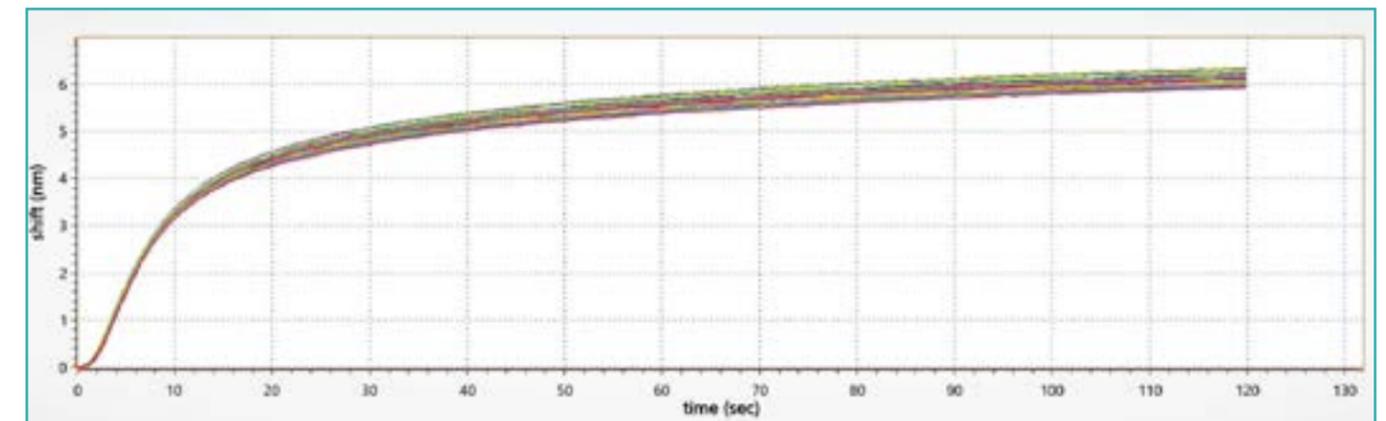


Figure 1:
Binding curve of human IgG to Pro A probes (1 µg/mL–2000 µg/mL in Q Buffer). Assay performed using standard protocol (400 rpm for 120 sec)

Regeneration to Save on Consumables

Pro A probes can be regenerated using the Gator™ GatorOne 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 A 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.

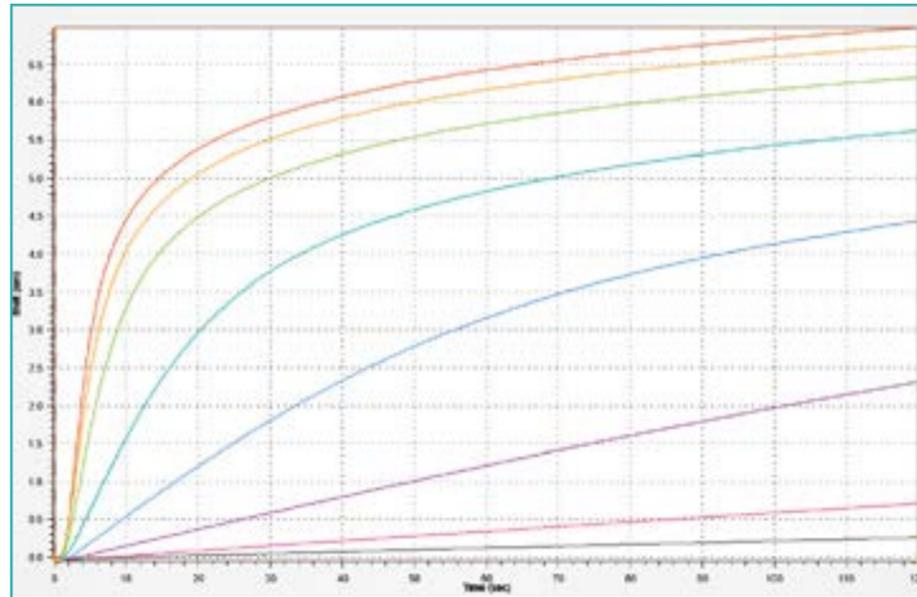


Figure 2:
50 consecutive measurements of human IgG (300 µg/mL in Q Buffer) on the same Pro A probe with regeneration. Assay performed using standard protocol (400 rpm for 120 sec). %CV of concentrations is 2.0%.

Ordering Information

Catalog No. 160001: Gator™ Protein A Probes

Gator™ Protein G (Pro G) Probes

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.

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)

Rapid Quantitation of Unknown Samples

Quantitation of crude and purified unknown samples can be performed using the Q assay preset on the Gator™ GatorOne 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–2 mg/mL. Probes can be regenerated in between samples to analyze an entire plate of samples in one run.

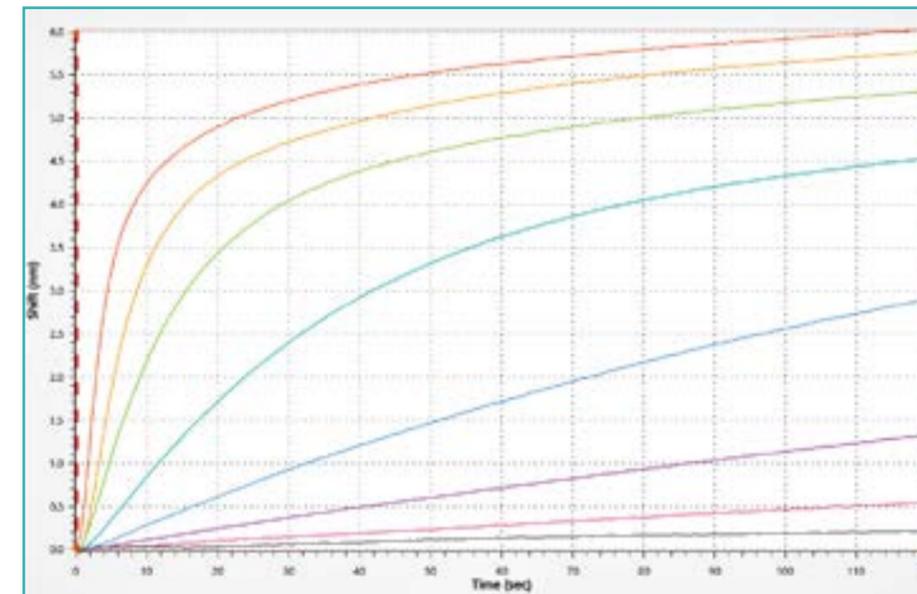


Figure 1:
Binding curve of mouse IgG to Pro G probes (1 µg/mL–2000 µg/mL in Q Buffer). Assay performed using standard protocol (400 rpm for 120 sec).

Regeneration to Save on Consumables

Pro G probes can be regenerated using the Gator™ GatorOne 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).

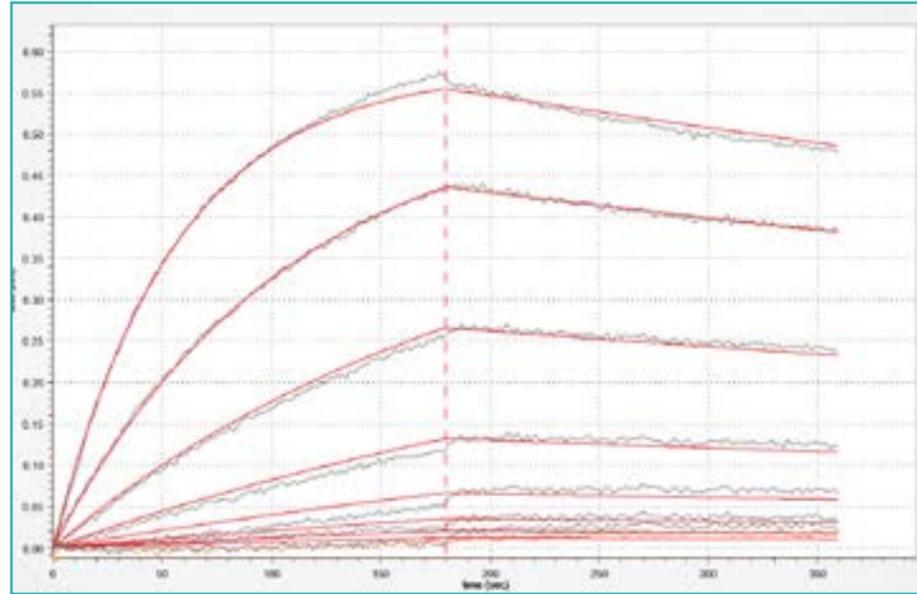


Figure 2:
A kinetics assay was performed with Pro G probes by loading mouse anti-fluorescein (5 µg/mL in K Buffer; 1000 rpm) followed by association and dissociation of a range of concentrations of fluorescein-BSA (30 nM–0.3 nM in K Buffer; 1000 rpm). Shown is the kinetics global-fit with best-fit lines 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	9.94×10^{-4}	5.14×10^5	1.93
Assay 2	8.73×10^{-4}	5.15×10^5	1.70
Assay 3	7.90×10^{-4}	4.95×10^5	1.60
Assay 4	7.41×10^{-4}	4.74×10^5	1.57
Assay 5	7.90×10^{-4}	4.58×10^5	1.72
Assay 6	7.85×10^{-4}	4.50×10^5	1.74
Assay 7	7.51×10^{-4}	4.45×10^5	1.69
Assay 8	7.42×10^{-4}	4.53×10^5	1.64
Assay 9	7.35×10^{-4}	4.32×10^5	1.70
Assay 10	7.24×10^{-4}	4.20×10^5	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.

Ordering Information

Catalog No. 160006: Gator™ Protein G Probes

Gator™ Protein L (Pro L) Probes

Overview

Gator™ Protein L (Pro L) Probes are useful in measuring the concentration and kinetics of antibodies. Specifically, Protein L binds specifically to certain subtypes of kappa light chain, including human VκI, VκIII and VκIV subtypes, and mouse VκI subtype. As there is no heavy chain involved in binding interaction, Protein L can bind to all antibody classes (IgG, IgM, IgA, IgE and IgD), as well as single chain variable fragments (scFv) and Fab fragments. These probes can be regenerated and reused for multiple experiments.

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)

Rapid Quantitation of Unknown Samples

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

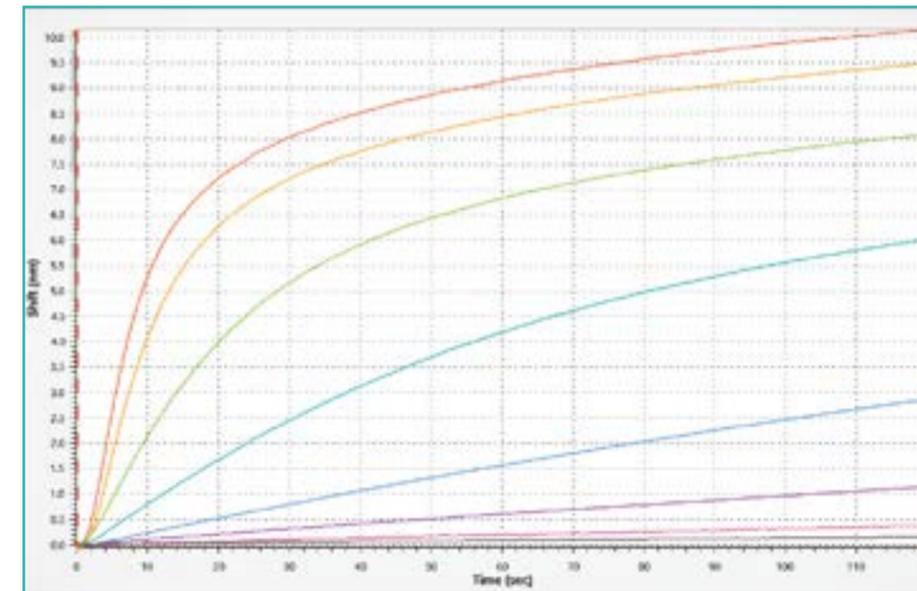


Figure 1:
Binding curve of human IgG to Pro L probes (1 µg/mL–700 µg/mL in Q Buffer). Assay performed using standard protocol (400 rpm for 120 seconds).

Regeneration to Save on Consumables

ProL probes can be regenerated using the Gator™ GatorOne 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 L 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.

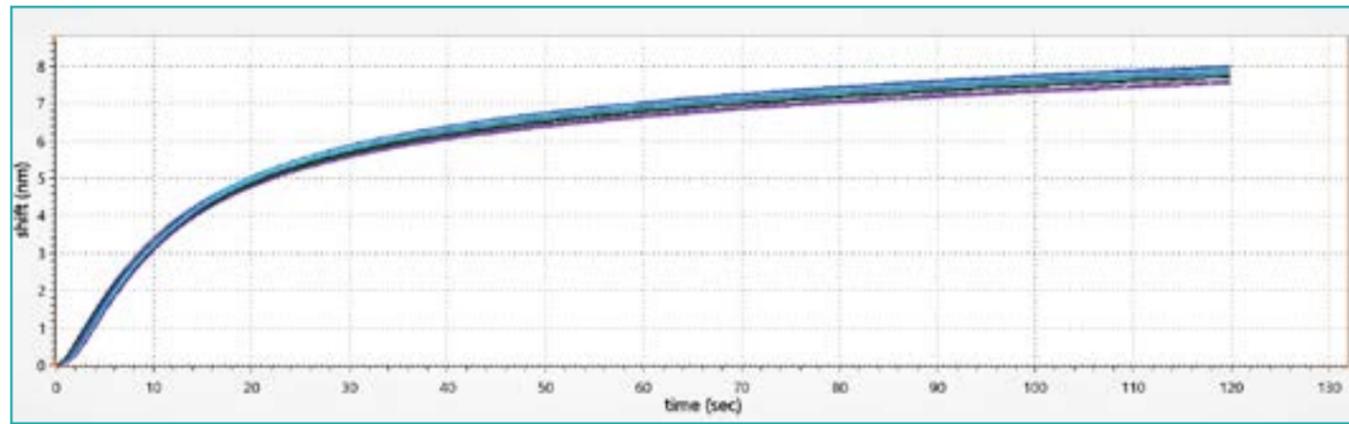


Figure 2:
40 consecutive measurements of human IgG (300 µg/mL in Q Buffer) on the same Pro L probe with regeneration. Assay performed using standard protocol (400 rpm for 120 sec).

Ordering Information

Catalog No. 160007: Gator™ Protein L Probes

Gator™ Anti-Human Fc (HFC) Probes

Overview

Gator™ Anti-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.

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

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 µg/mL–300 µg/mL.

Known Concentration (µg/mL)	Average Calculated Concentration (µg/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).

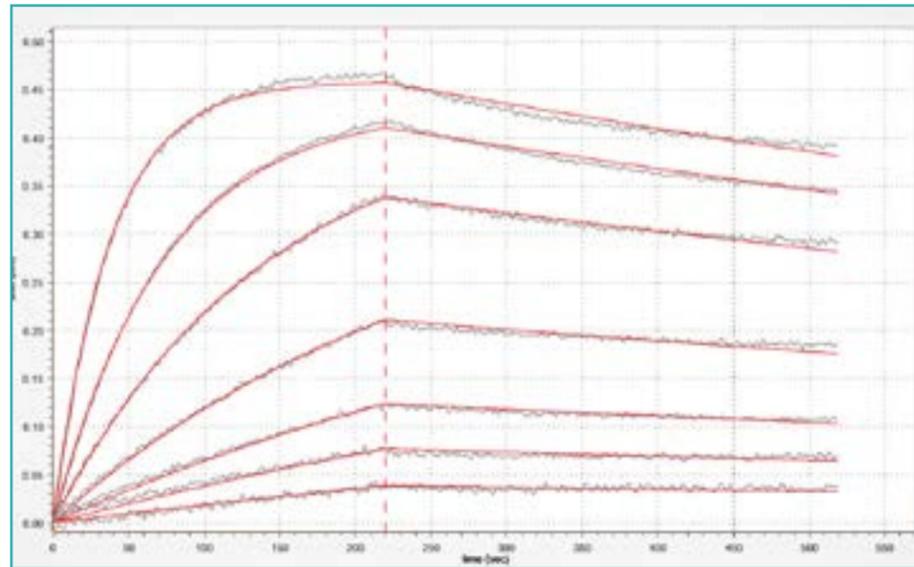


Figure 1:
Human IgG (5 µg/mL in K Buffer) was loaded onto HFC probes followed by association and dissociation of a range of concentrations (0.3–100 nM in K Buffer) of fluorescein-BSA. Global-fit analysis was performed (best-fit lines in red), and the affinity is 1.68 nM.

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

Table 2:
Human IgG (5 µg/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.

Ordering Information

Catalog No. 160003: Gator™ Anti-Human Fc (HFC) Probes

Gator™ Anti-Human FAB

Overview

Gator™ Anti-Human FAB biosensors are useful for the quantitation or kinetic characterization of hIgG antibodies, hIgG F(ab) fragments, or hIgG F(ab')₂ fragments and their respective antigens. The proprietary surface chemistry allows for high-capacity immobilization of hIgG antibody or antibody fragments expressing the CH1 domain.

Following immobilization, users can quantify hIgG antibody/antibody fragments of interest or determine the k_{on} , k_{off} , and K_D of the binding interaction between hIgG/human antibody fragments and their antigen.

General Applications

Quantitation and/or Kinetic studies of human antibody or human F(ab), or F(ab')₂ fragments.

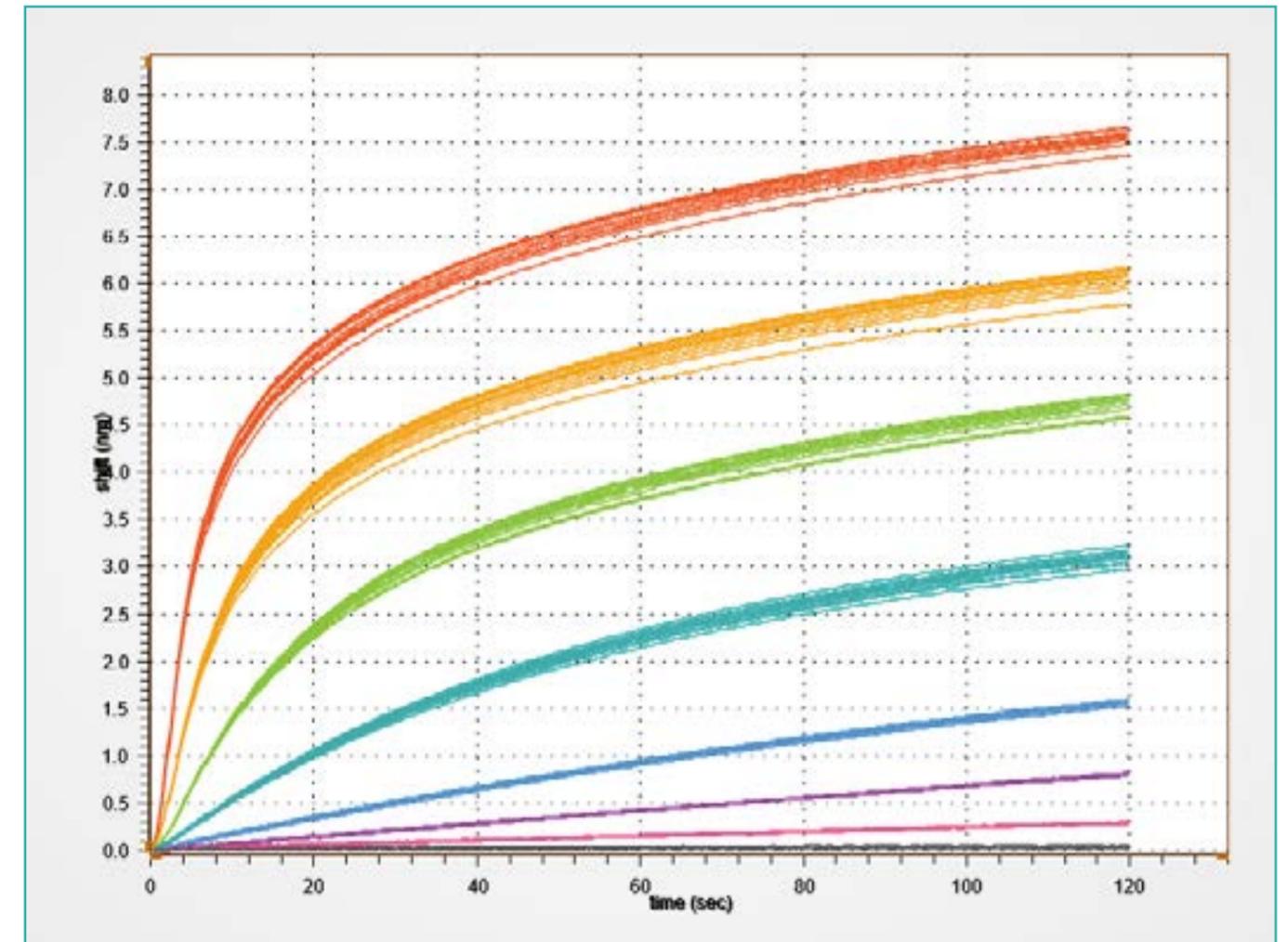


Figure 1:
Following a 10 min 1000 rpm pre-wet in our quantitation buffer, hIgG was loaded onto Anti-Human FAB biosensors over a range of concentrations (0.1 - 3000 µg/mL) for 20 rounds of regenerations.

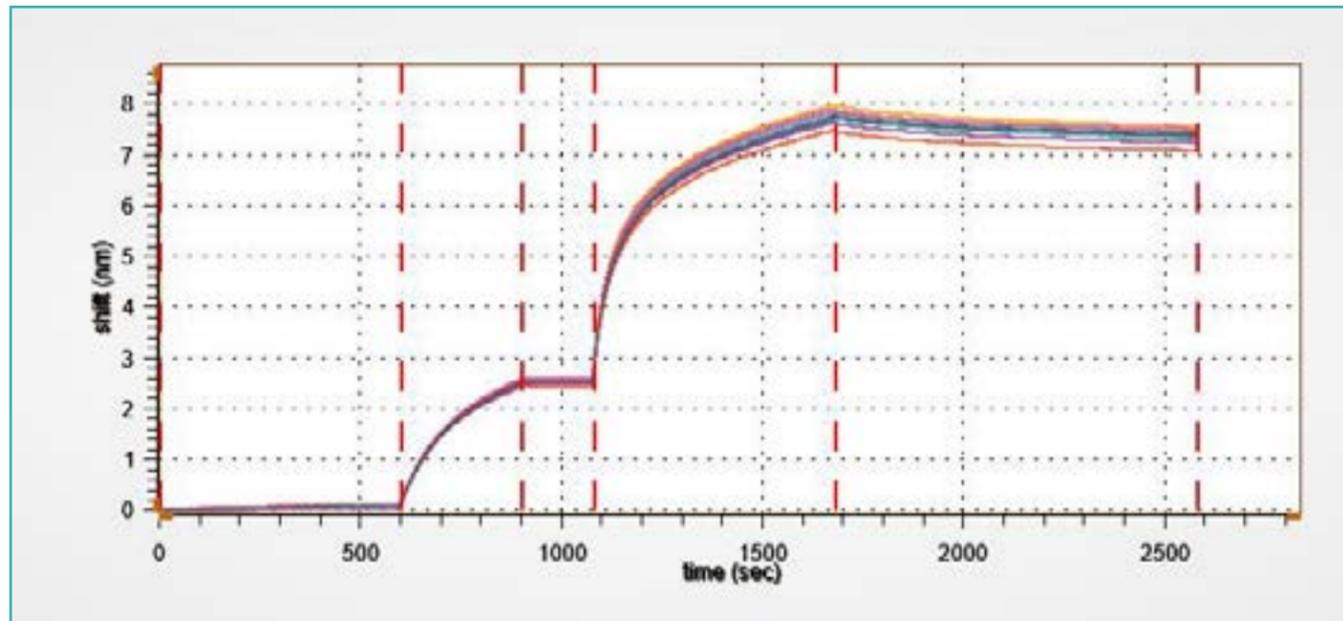


Figure 2: Following pre-wet equilibration (10 min at 1000 rpm) in K Buffer, hlgG was loaded onto Anti-Human FAB biosensors, then exposed to association and dissociation of 200 nM polyclonal goat anti-human F(ab')₂ antibody. Global-fit analysis using Gator™ GatorOne Software for the hlgG binding interaction with anti-hlgG F(ab')₂ resulted in $K_D = 6.47 \times 10^{-10}$ M.

Tips for Optimal Performance

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

Ordering Information

Catalog No. 160013: Gator™ Anti-Human FAB Probes

Gator™ Anti-His Probes

Overview

Gator™ Anti-His Probes are used to capture polyhistidine-tagged (His-tagged) proteins for quantitation (Q), kinetics (K) and quantitation-kinetics-regeneration (QKR) assays. Anti-His probes come pre-coated with a high-affinity, monoclonal antibody that recognizes His-tags on both the C- and N-termini. These probes can be used to measure purified samples in buffer as well as proteins in supernatant or crude mixtures.

General Applications

1. Quantitation of His-tagged protein
2. Kinetics assay of His-tagged protein
3. Determination of concentration and kinetics of interaction of His-tagged protein in one run (QKR)

Regeneration to Save on Consumables

Anti-His probes can be regenerated using the Gator™ GatorOne 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 Anti-His 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.

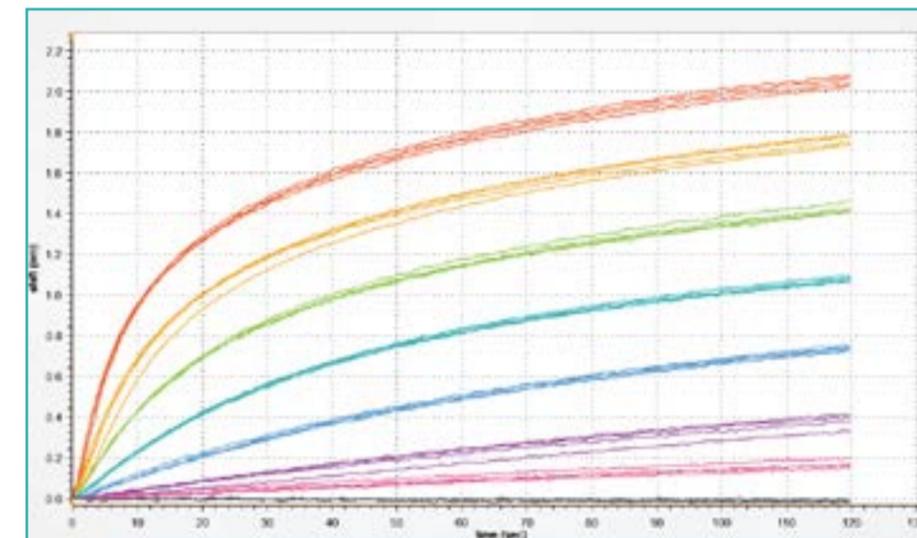


Figure 1: Repeated measurements of His-tagged human serum albumin (His-HSA; 0–500 µg/mL in Q Buffer) on the same Anti-His Probe following regeneration. %CV is <5% for all concentrations.

Rapid Quantitation of Unknown Samples

Quantitation of crude and purified unknown samples can be performed using the Q assay preset on the Gator™ GatorOne Software. For accurate results, make a standard curve of known concentrations in the same buffer as the unknowns. For the most accurate quantitation results, fresh probes are recommended.

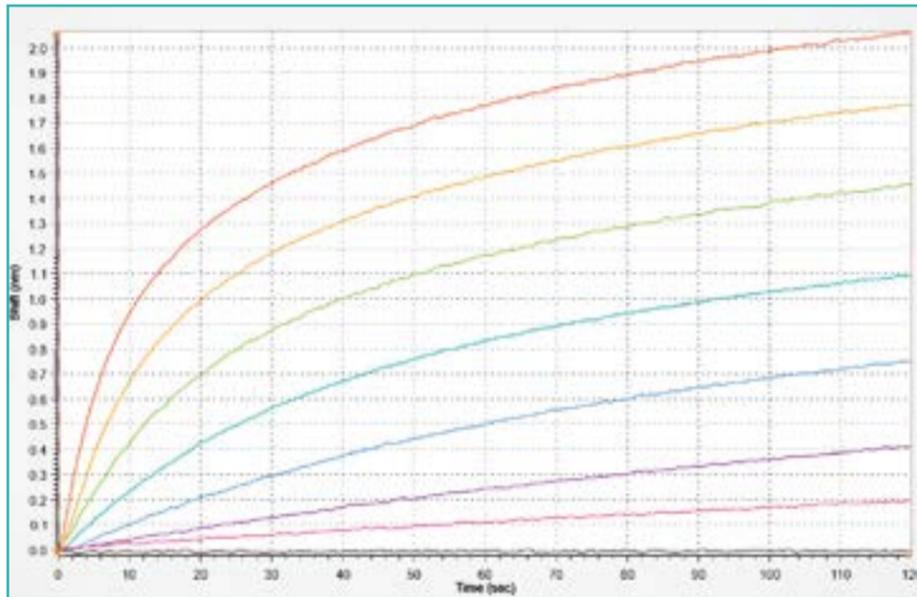


Figure 2:
Standard curve of His-HSA (12.5–500 µg/mL in Q Buffer) bindings to Anti-His probe. Assay was performed at 400 rpm for 120 sec.

Kinetics Study of His-tagged Proteins with Binding Partners

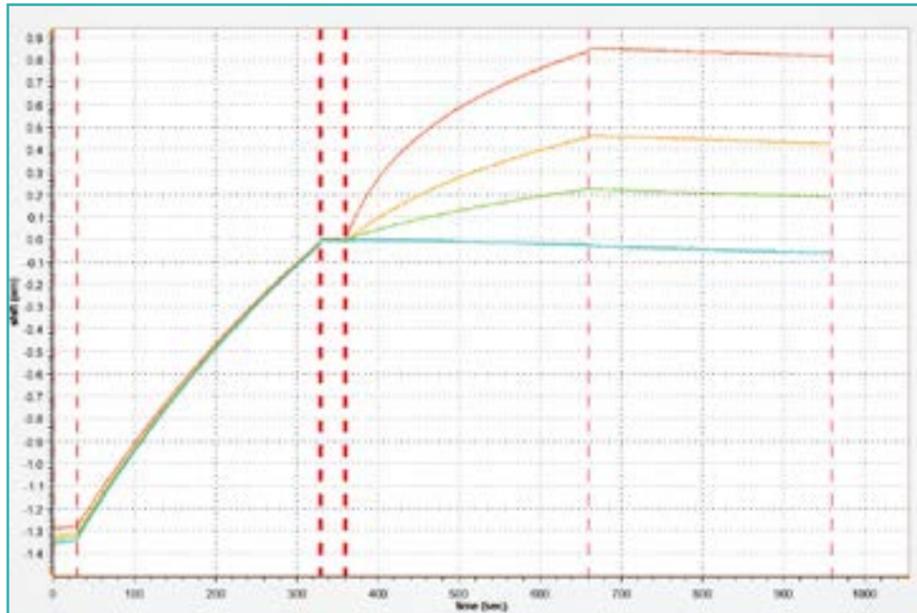


Figure 3:
Loading of His-LAG3 (100 nM diluted in K Buffer) onto Anti-His probes followed by association and dissociation of FGL1 at various concentrations (0–300 nM in K Buffer). Global-fit analysis found the affinity to be 8 nM.

Ordering Information

Catalog No. 160009: Gator™ Anti-His Probes

Gator™ Amine Reactive Probes

Overview

Gator™ Amine Reactive (AR) Probes are useful for determining kinetics of molecular interactions between protein and analyte. The AR probe surface comes pre-coated for the covalent attachment of a purified protein. The coupling process occurs through an EDC-activated amide bond between the reactive amine on the protein and the carboxy terminated probe surface. AR probes are well suited for high-affinity interactions due to the covalent immobilization.

General Applications

1. Kinetics assays of protein-protein interactions
2. Indirect quantitation assays
3. Epitope binning

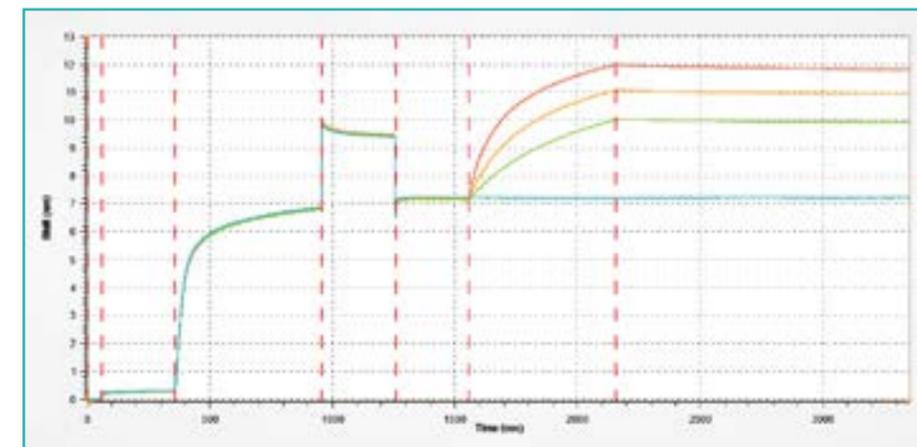


Figure 1:
Amine Reactive Probe loaded with mouse IgG

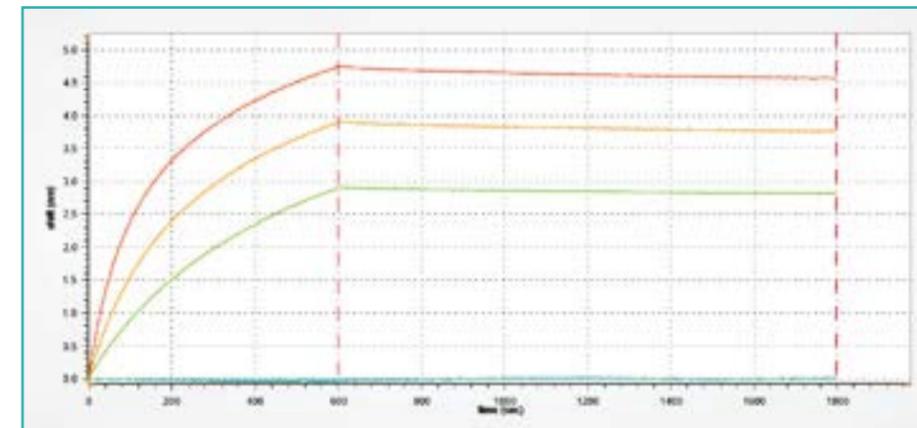


Figure 2:
Association and dissociation kinetics of anti-mFc antibody in a two-fold series dilution from 12.5 nM–50 nM. 2.5 µg/mL mIgG was loaded.

k_{obs} (1/s)	4.04×10^{-3}
k_{off} (1/s)	2.66×10^{-5}
k_{on} (1/M*s)	1.37×10^5
K_D (nM)	1.93×10^{-10}

Ordering Information

Catalog No. 160008: Gator™ Amine Reactive Probes

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