



# Discover Next-gen Biolayer Interferometry with Gator Prime

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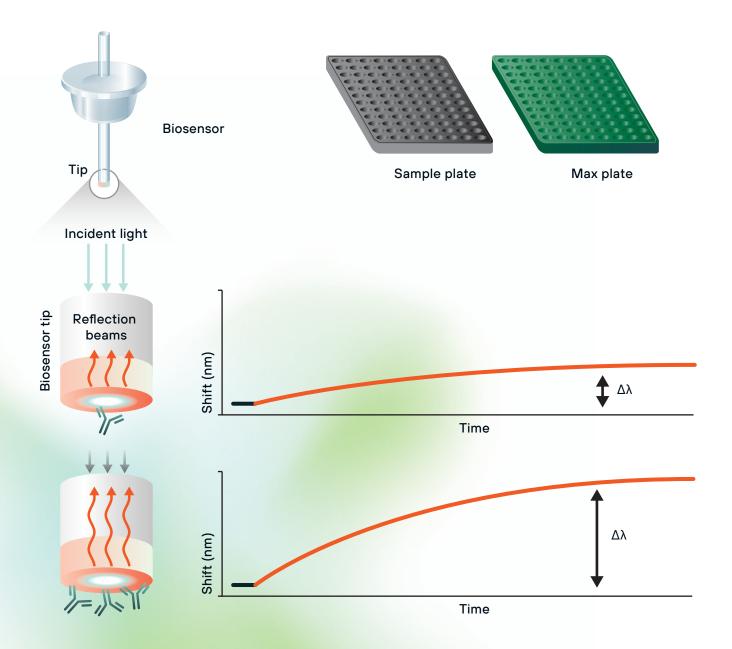




## What is BLI?

Biolayer interferometry (BLI) is a label-free detection method based on reflection of white light from the surface of a biosensor tip.

It analyzes the changes in interference pattern of white light reflected from the tip when biomolecules bind to it. This change is recorded in real time and is expressed as nanometer shift. It is proportional to the number and size of biomolecules bound to the tip.



## One Tool. Many Answers.

The Gator biolayer interferometry system is ideally suited for studying biomolecular interactions in academic research and to support multiple stages of therapeutic development in biopharma.

The Gator® Prime system is designed for real-time analysis of biomolecules and can be widely applied in antibody screening, quantitation and epitope binning. It also enables AAV and other viral particle analytics.

#### Early discovery

Antibody titer determination

Yes/no binding to target antigen

Isotyping

Epitope binning

Cross-reactivity testing

Assay development

Off-rate ranking

Binding constant determination



Gator Prime

Gator Total Solution

Gator biosensors and consumables

Gator One software

#### Early development

Lead optimization

Lead characterization

Detailed kinetic characterization

Epitope binning

Affinity maturation

#### Lead antibody

Binding kinetics

Activity assay

Stability study

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### Main Features

# Standard Biolayer Interferometry (BLI) System with Gator Prime

#### Highlights



8 Spectrometers enable high frequency parallel measurement of up to 168 samples.



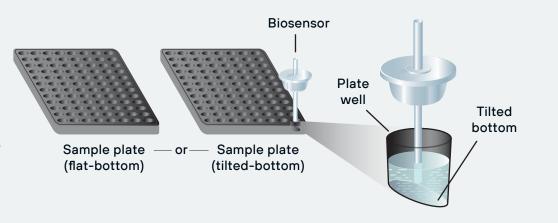
Single sample plate enables automated data acquisition for 168 samples per batch.

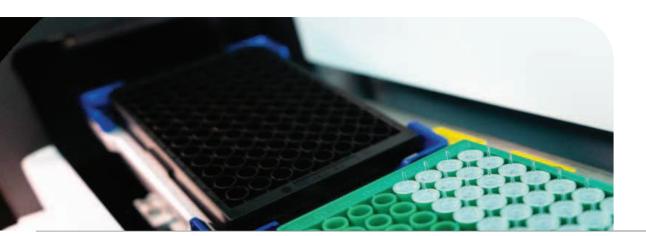


With Gator Bio next-gen biosensors, the Gator Prime system provides accurate, high sensitivity data

The Gator Prime instrument accommodates one 96-well microplate either tilt-bottom or flat-bottom format.

Another plate is reserved for biosensors placement.





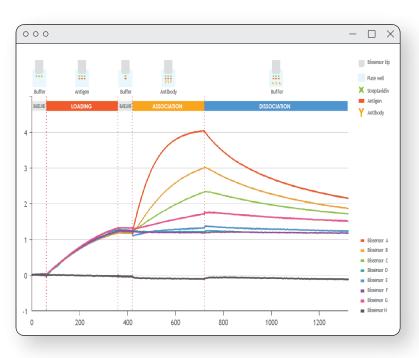
## Kinetic Characterization

Get a wide array of probes for the kinetic characterization with Gator Prime.

The Gator® system can be used to determine kinetics of a drug molecule binding to its target. The system enables quick measurements of association rate  $(k_{on})$ , dissociation rate  $(k_{off})$  and equilibrium dissociation constant  $(K_D)$  of antigen-antibody interactions with or without the use of labeled reagents.

Gator Prime can be used for primary kinetic screening of antibody libraries in different crude media to determine the off-rate ranking as well as complete binding characterization of a purified antigen-antibody binding pair.

A wide array of probes are available for the kinetic characterization of biological samples. In general, Gator Bio biosensors are very versatile and easily regenerable and can be used multiple times. This capability allows the user to set up multiple real-time binding assays with proper controls and blank sensors to get high-quality binding data.



Kinetic analysis of a purified antigen-antibody pair using Gator Bio Streptavidin biosensors.

#### Highlights



Eight different binding reactions simultaneously



Binding constant determined within 10 minutes



Easy to customize assay and fine-tune concentration ranges of analyte to get accurate binding constants



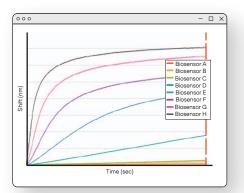
Wide range of biosensor choices to determine binding kinetics several ways

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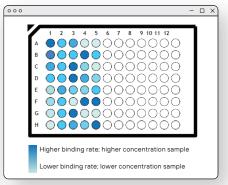
# Antibody Titer using Protein A Biosensors

High-sensitivity Gator Bio Protein A biosensors allow the effective and specific capture of any Fc-tagged protein that binds to protein A. The dynamic range for Gator Bio Protein A biosensors is from 25 ng/mL to 2 mg/mL in most commercially available media, allowing the analysis of a wide range of hybridoma supernatants with one simple setup.

Gator Bio Protein A biosensors can be regenerated multiple times with simple regeneration conditions.



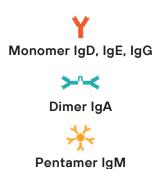
Standard curve for human IgG binding to Gator Prime Protein A biosensors

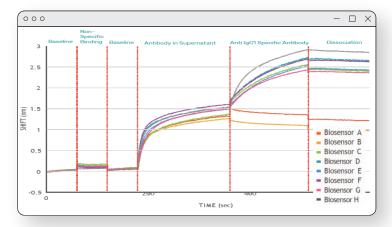


Heat map generated by Gator Prime software for human IgG concentration analysis using Gator Protein A biosensors

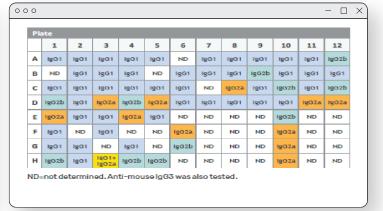
#### Antibody isotyping and subtyping

Immunoglobulins, i.e., antibodies, may be divided into five major classes: IgA, IgD, IgE, IgG, and IgM. Different species may have specific subclasses/isotypes of antibodies within these five major classes. These subclasses differ in the disulfide bonds linking the two heavy chains of the antibody in the constant region. For example, among IgG in mice, there are IgG1, IgG2a, IgG2b, and IgG3. A key Gator Bio application is identifying isotypes (IgG1, IgG2a, etc.) with Gator Bio Human Fc (HFC) and Mouse Fc (MFC) biosensors. Using Gator software, users may easily and definitively identify the presence of antibody subtypes in crude samples, such as hybridoma supernatants.





Hybridoma supernatant isotyping using Gator Mouse Fc biosensors



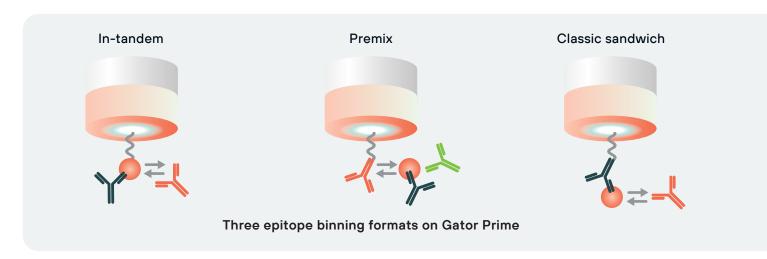
90-well plate with unknown isotypes of mouse IgG hybridoma supernatant characterized using Gator Mouse Fc biosensors

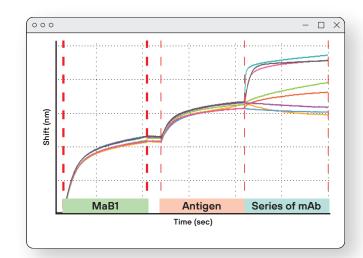
# **Epitope Binning**

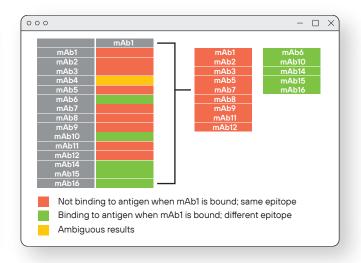
When an antibody binds to an antigen, it is not binding to the entire protein sequence. Instead, the antibody binds to a segment of the antigen that is approximately five or six amino acids in length. As such, a typical protein contains many different epitopes that antibodies can recognize and bind.

In epitope binning, antibodies are tested in a pairwise combinatorial manner, and those that compete for the same binding region of the antigen are grouped together into bins. An epitope bin is a relative concept based on the epitopes represented within the panel of mAbs being tested.

Gator® Prime monitors real-time binding and gives a clear picture of whether a particular antigen-antibody pair is in the same epitope bin depending on changes in the signal or nanometer shift. Several Gator probes (Human Fc, Mouse Fc, Anti-His, etc) are available for epitope binning. The experiment can be set up to run in-tandem, premix and classical sandwich formats. The probes and materials are reusable.







12 x 12 mAb competition matrix performed in less than 5 hours

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# Specifications

#### Gator Prime



General	
Detection	Biolayer Interferometry
Sample Microplate	96-well format
Sample type	Proteins, antibodies, peptides, nucleic acids, liposomes, viruses, and small molecules
Maximum sample capacity	168
Software	Integrated
Simultaneous reads	8
Spectrometers	8
Acquisition rate	2, 5, and 10 Hz
Dimension - H x W x D (cm)	50 x 67 x 31
Weight	35 kg
Orbital flow	Static, 100 - 2000 rpm
Analysis temperature range	Ambient plus 4°C to 40°C
Kinetics	
Analysis time	Real-time kinetic binding from 5 min to 4 hr
Baseline noise (RMS)	≤ 4 pm
Baseline drift	≤ 0.12 nm/hr
Association rate (k <sub>on</sub> )	10¹ to 10 <sup>7</sup> M⁻¹ s⁻¹
Dissociation rate (k <sub>off</sub> )	10 <sup>-6</sup> to 10 <sup>-1</sup> s <sup>-1</sup>
Affinity constant (K <sub>D</sub> )	1 mM - 10 pM
Molecular weight	>150 Da
Quantitation	
Analysis time	8 samples in 2 min
Quantitation range (Protein A Biosensor)	0.02 – 2000 μg/mL
Quantitation precision (Protein A Biosensor)	CV < 10%
Epitope binning	
Analysis time	Up to 12x12 in 5 hr
Pairwise fashion	In-tandem, classic sandwich and pre-mix
Binning capacity	12x12

# Gator Probes

## Applications & Specifications

Gator Probes	Applications		Cuantitation Dynamic range (µg/mL)			
NTIBODY BIOSENSC	PRS					
Pro A	lgG titer	•		•	0.02 - 2000	•
Pro G	lgG titer	•		•	0.02 - 2000	
Pro L	lgG Titer using kappa-light chain	•		•	0.02 - 2000	
HFC	Human IgG characterization by human IgG Fc capture		•	•		•
► HFC Gen II	Second-generation HFC probes with higher affinity capture and better regenerability	•	•	•	0.3 - 6000	
MFC	Mouse IgG characterization by mouse IgG Fc capture	•	•	•	0.02 - 6000	•
Anti-Rabbit Fc	Rabbit IgG characterization by rabbit IgG Fc capture	•	•	•	0.05 - 4000	
Anti-FAB	F(ab), F(ab)2 characterization by CH1 capture	•	•	•	0.3 - 3000	
⊭ lgM	Human IgM titer/characterization	•	•		0.4 - 300	
P Anti-VHH	Camelid anti-VHH characterization	•	•		0.05 - 10	
PURIFICATION TAGS						
Anti-His	Captures C- and N-terminal 6-His and 8-His tagged proteins	•	•	•	~1 - 100	•
Ni-NTA kit	Ni-NTA capture surface for purified His-tagged proteins	•	•	•	~1 - 100	
Strep-Tactin XT	Captures win-strep-tagged proteins (seq: SAWSHPQFEKGGGSGGSGGSAWSHPQFEK)	•	•	•	~0.02 - 20	
Anti-GST	Captures GST-tagged proteins	•	•	•	0.5 - 300	
Anti-FLAG	Captures FLAG-tagged proteins		•			
STREPTAVIDIN SUITE						
SA	Streptavidin surface. Captures biotinylated molecules		•	•		
SA XT	High sensitivity SA probe for low Mw (>1 kDa) and high Mw (<2MDa) analytes		•	•		
* SMAP	High sensitivity SA for small molecule and small peptide analytes.		•			
FlexSA kit	Re-activatable SA biosensor kit		•			
CELL & GENE THERAF	PΥ					
AAVX/AAV9	Measures intact AAV viral particle titer	•	•		7E <sup>9</sup> - 1E <sup>14</sup> vp/ml	
HS AAVX/AAV9 kit	Measures low concentration intact AAV viral particle titer	•			1E <sup>7</sup> - 1E <sup>9</sup> vp/ml	
• AAV Ratio kit	Determine AAV empty/full capsid ratio	•			5 - 100% full	
Anti-PEG	Captures PEGylated proteins/LNPs		•			
JSER-CUSTOMIZED (	CHEMISTRIES					
AR	Amine coupling surface ready for EDC-NHS coupling		•			
APS	APS surface for hydrophobic ligand capture		•			
P Custom	Customized to your specifications	•	•	•	Varies	Va

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