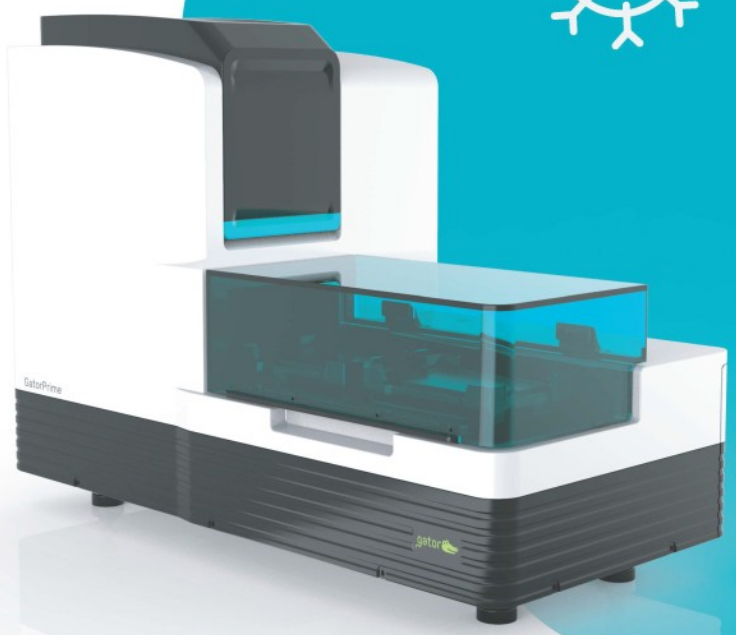
Gator® Prime

Label free analysis



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A tool for drug discovery

Based on biolayer interferometry (BLI) technology, the GatorPrime system enables real-time measurements to support the analysis of biological molecules at different stages of therapeutic development. The Gator instrument measures the interference pattern caused by changes in the amount of protein bound to the probe tips. This is useful for studying protein-protein interactions: off-rate screening, binding constant determination, yes/no binding to target antigens, affinity maturation and epitope binning.

EARLY DISCOVERY	EARLY DEVELOPMENT	LEAD ANTIBODY
• Antibody titer determination	• Lead optimization	• Binding kinetics
• Yes/no binding to target antigen	• Lead characterization	• Activity assay
• Isotyping	• Detailed kinetic characterization	• Stability study
• Epitope binning	• Epitope binning	
• Cross-reactivity testing	• Affinity maturation	
• Assay development	• FcR/FcRn binding	
• Off-rate ranking		
• Binding constant determination		

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The GatorPrime system

The GatorPrime system includes instrumentation, software and a selection of probes. The integrated software combines acquisition and analysis to enable setup from start to finish. Many ELISA assays, including antibody titer determination and isotyping, can be implemented readily on the GatorPrime system.

Gator™ Probe	Function	Application	Dynamic range	Regeneration
Protein A	Binds IgGs of various species including human and mouse	Q	0.02 - 2000 µg/mL	Yes
Protein G	Binds IgGs of various species including human and rat	Q	0.02 - 2000 µg/mL	Yes
Protein L	Binds IgGs of various species through the kappa light chain	Q	0.1 - 2000 µg/mL	Yes
Human Fc	Immobilize human Fc-fusion protein or human IgG for quantitative or kinetic analysis	Q/K/QKR/EP	0.05 - 300 µg/mL	Yes
Mouse Fc*	Immobilize mouse Fc-fusion protein or mouse IgG for quantitative or kinetic analysis	Q/K/QKR/EP	0.02 - 2000 µg/mL	Yes
Anti – FAB*	Binds F(ab), F(ab')2, Fc receptor, and full-length Human IgG	Q/K/QKR/EP	0.3 - 3000 µg/mL	Yes
Anti – His	Binds His-tagged proteins	Q/K/QKR/EP	Protein-dependent	Yes
Ni-NTA	Tris-NTA and charged with Ni^{2+} ions binding to His-tagged proteins	Q/K/QKR/EP	Protein-dependent typically 0.25 – 1000 ug/mL	Yes
Streptavidin (SA)	Binds biotinylated and Avi-tagged biomolecules	K/EP	Protein-dependent	No
Small Molecule, Antibody, and Protein (SMAP)	Binds biotinylated and Avi-tagged biomolecules and subsequent binding of small molecules and proteins	K	>150 Da	No
APS (Aminopropylsilane)	Binds hydrophobic proteins	K	Protein-dependent	No
Amine – Reactive	Covalently attach amine group of proteins using EDC/NHS	K/EP	Protein-dependent	No
AAVX*	Binds serotypes AAV1-AAV8 and AAV10	Q	10^9 – 10^{13} vp/mL	Yes
Flex SA	Binds biotinylated and Avi-tagged biomolecules with reactivable sensor surface	K	Protein-dependent	Reactivable
Custom - Made	Custom made biosensors for your specific applications (SARS-CoV-2 RBD, Anti-Rabbit, Anti-Rat and Anti-FLAG)	Varies	Varies	Varies

*For the best performance, it is recommended to regenerate the probes using Regeneration Buffer - No Salt (Cat No. 120063) prior to use.

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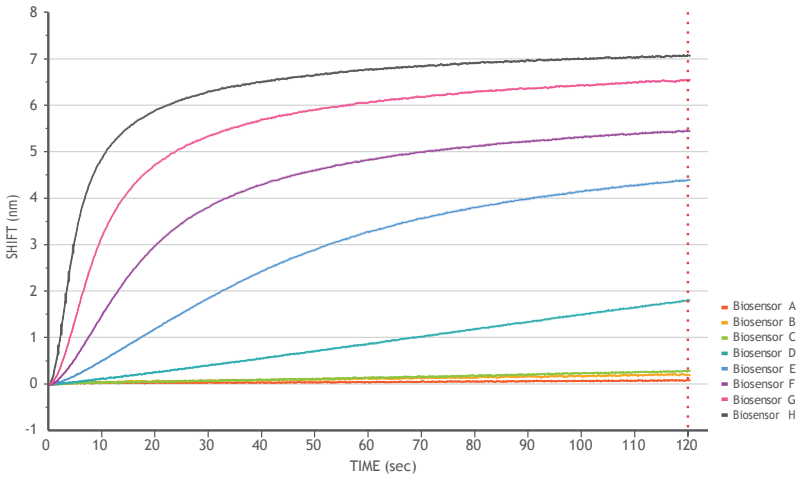
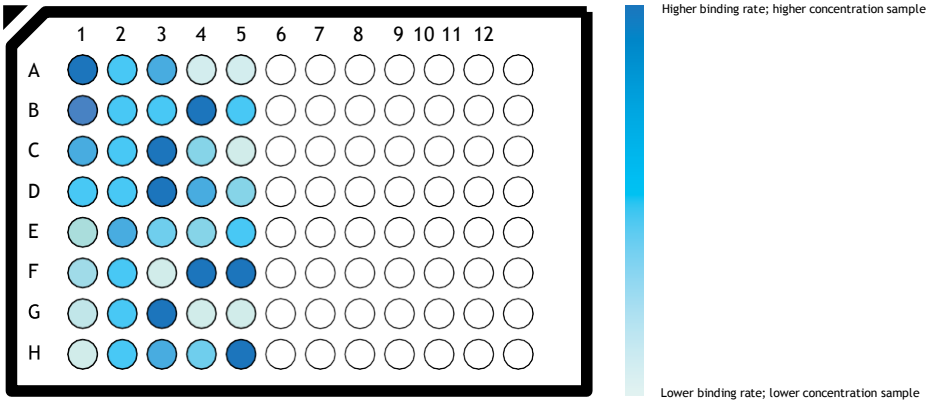
Antibody titer using Gator Bio Protein A biosensors

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 more than 20 times with simple regeneration conditions, and the same biosensors can be used over a period of multiple days.

Standard curve for human IgG binding to GatorPrime Protein A biosensors



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



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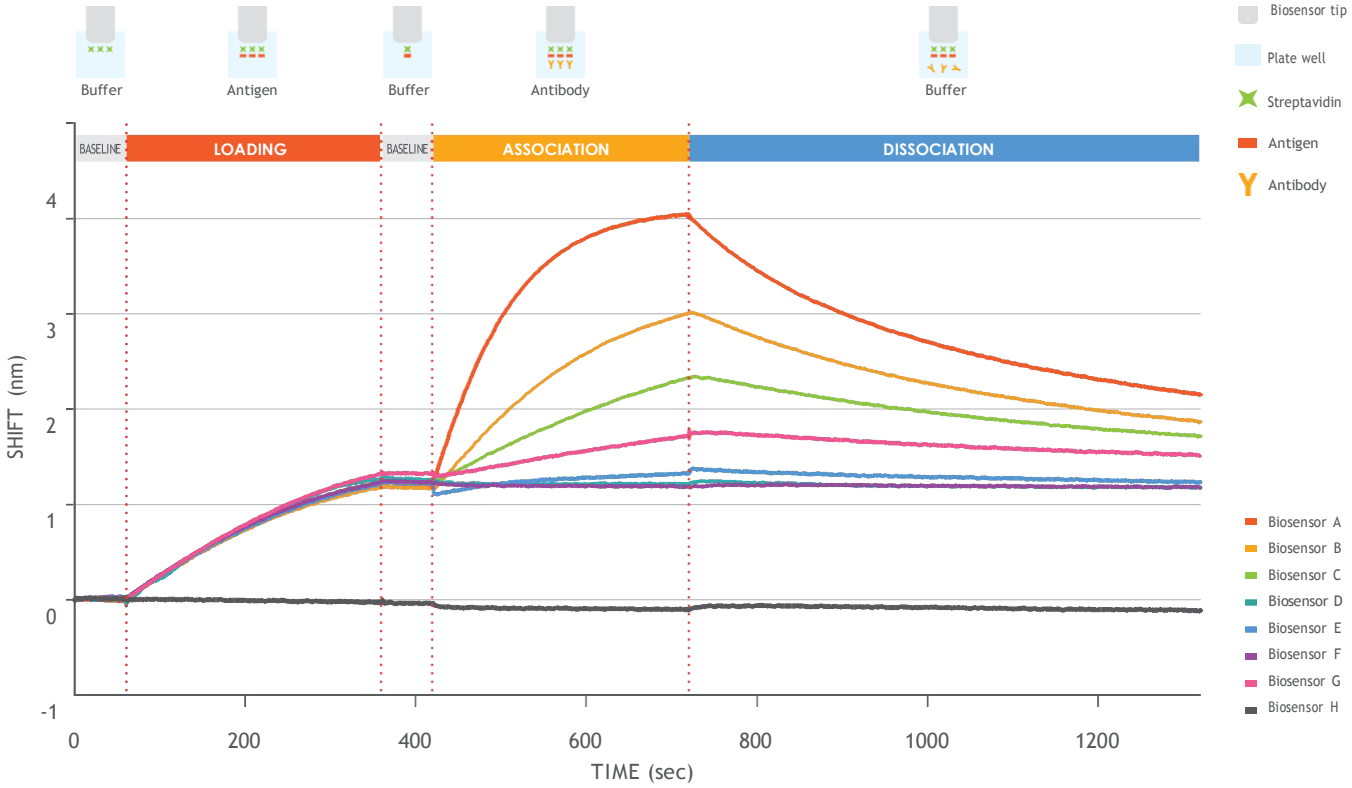
Kinetic characterization using the GatorPrime system

The Gator system can be utilized 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.The Gator system 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 is available for the kinetic characterization of biological samples. In general, Gator Bio biosensors are very versatile and easily regenerable (except for the SA biosensors) 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 20 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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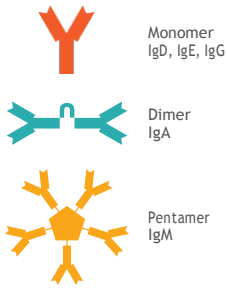
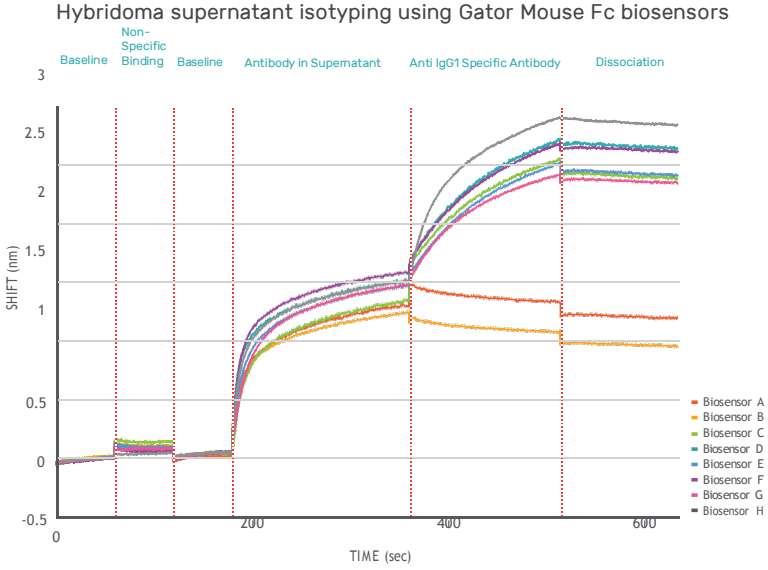
Antibody isotyping and subtyping with GatorPrime system

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 presenceof antibody subtypes in crude samples, such as hybridoma supernatants.



Hybridoma supernatant isotyping using Gator Mouse Fc biosensors



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

Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	IgG1	IgG1	IgG1	IgG1	IgG1	ND	IgG1	IgG1	IgG1	IgG1	IgG1	IgG2b
B	ND	IgG1	IgG1	IgG1	ND	IgG1	IgG1	IgG1	IgG2b	IgG1	IgG1	IgG1
C	IgG1	IgG1	IgG1	IgG1	IgG1	IgG1	ND	IgG2a	IgG1	IgG2b	IgG1	IgG2b
D	IgG2b	IgG1	IgG2a	IgG2b	IgG2a	IgG1	IgG1	IgG1	IgG1	IgG1	IgG2a	IgG2a
E	IgG2a	IgG1	IgG1	IgG2a	IgG1	ND	ND	ND	ND	IgG2b	ND	ND
F	IgG1	ND	IgG1	ND	ND	IgG2a	ND	ND	ND	IgG2a	ND	ND
G	IgG1	IgG1	ND	IgG1	ND	IgG2b	ND	ND	ND	IgG2a	ND	ND
H	IgG2b	IgG1	IgG1+ IgG2a	IgG2b	IgG2b	ND	ND	ND	ND	IgG2a	ND	ND

ND=not determined. Anti-mouse IgG3 was also tested.

Highlights

- Identification of antibody isotypes even in crude samples
- High sensitivity
- Visual real-time binding results
- Regenerable biosensors to save time and cost/assay

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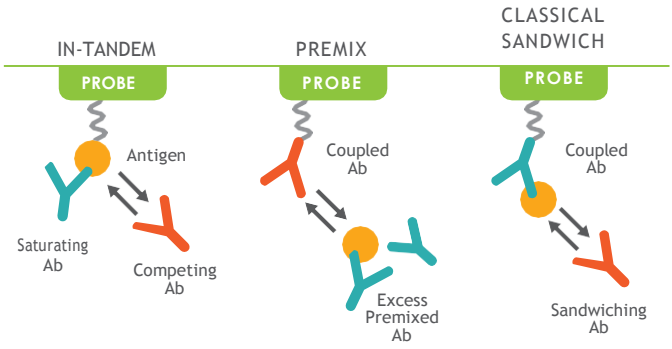
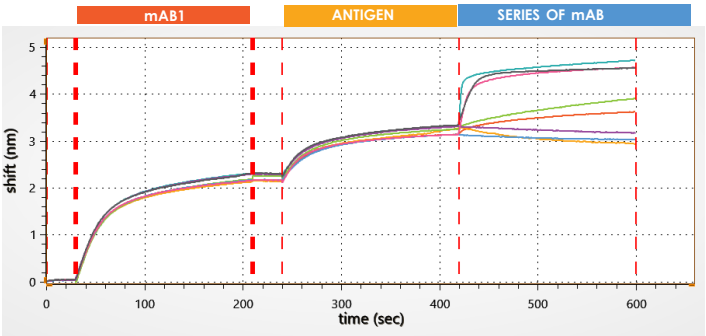
Epitope binning with GatorPrime system

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.

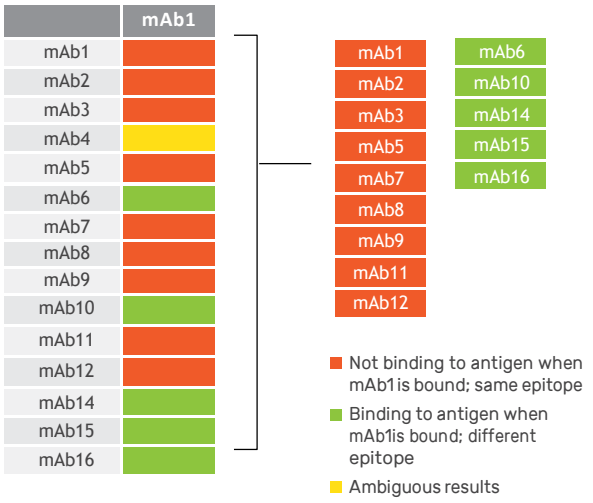


The GatorPrime system monitors real-time binding and gives a clear picture of whether a particular antigen-antibody pair is in the same epitope bin depending on changes in the signal or nanometer shift. Several Gator Bio probes (Human Fc, Mouse Fc, Anti-His, etc) are available for epitope binning. The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

Epitope binning binding curve using in-tandem assay



Epitope binning against supernatant



The results shown here suggest two different epitope groups.

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GENERAL

Detection	Biolayer interferometry
Number of channels	8
Data collection rate	2 Hz, 5 Hz, 10 Hz
Orbital flow	0 – 1,500 rpm
Instrument	460 mm x 670 mm x 320 mm (H x W x D) 30 kg
Electrical	110V – 220V 5A

SOFTWARE

- Integrated data acquisition and analysis
- QKR—quantitation, kinetics and regeneration in one run
- Epitope binning setup and analysis
- Smart search and preview of results
- Data analysis templates for multiple binding models
- Real-time display of quantitative results and heat map
- Allows initiation of next assay while current one is in progress
- Operating system: Windows 10 Professional 64-bit

ANALYSIS

Sample types	Proteins, antibodies, peptides, small molecule, DNA,RNA, liposomes, cells, viruses, etc.
Work flow	• 2 x 96-well plates, in tilted or flat position • Up to 8 samples in parallel, up to 96 samples per 96-well plate
Types of analysis	• Yes or no binding • Relative and absolute concentration • Kinetics and affinity • Epitope binning

QUANTITATION

Time to Result	• IgG concentration in 30 seconds for 8 samples • 26 minutes for 96 samples*
Linear range for Gator Protein A biosensor	0.02 – 2,000 µg/mL

*Protocol and instrument dependent

KINETICS

Analysis time	Real-time kinetic binding from 1 minute to 4 hours
Association rate k_{on}	10^{1} – 10^{7} M^{-1}/s
Dissociation rate k_{off}	10^{-6} – $10^{-1}/s$
Affinity constant K_D	10 pM – 1 mM
Binding curve baseline noise	< 4 pm (RMS)
Binding curve baseline drift	< 120 pm/hour (RMS)

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