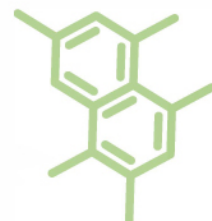
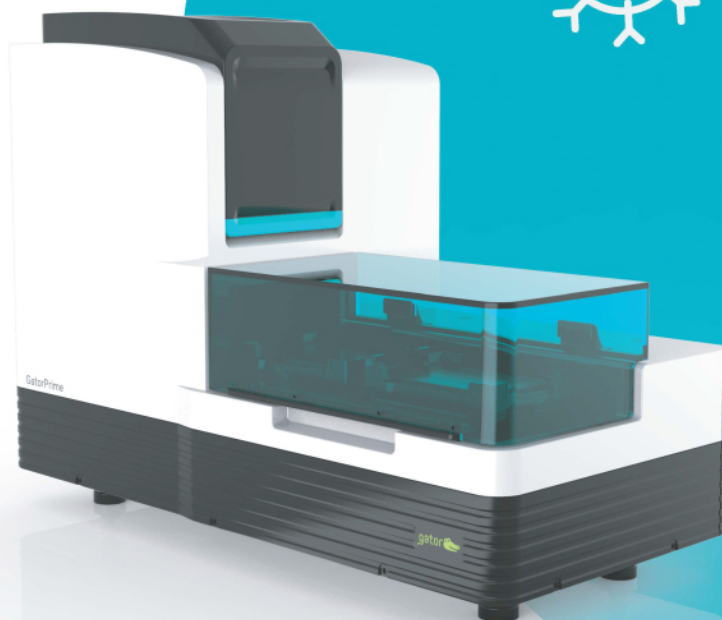
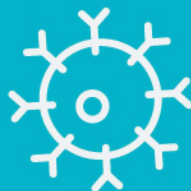




GatorPrime

Label free analysis



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

- Antibody titer determination
- Yes/no binding to target antigen
- Isotyping
- Epitope binning
- Cross-reactivity testing
- Assay development
- Off-rate ranking
- Binding constant determination

EARLY DEVELOPMENT

- Lead optimization
- Lead characterization
- Detailed kinetic characterization
- Epitope binning
- Affinity maturation
- FcR/FcRn binding

LEAD ANTIBODY

- Binding kinetics
- Activity assay
- Stability study



The GatorPrime system

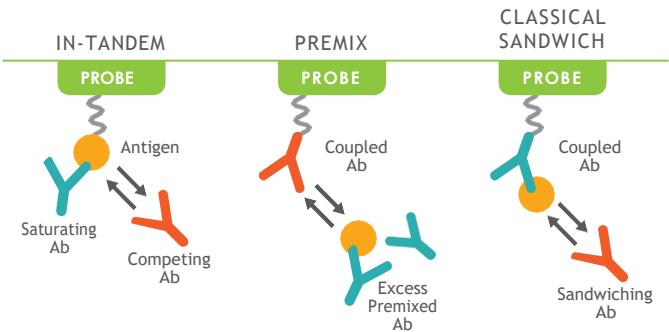
The GatorPrime system includes instrumentation, software and a selection of probes. The integrated software combines acquisition and analysis to enable setup from start to finish. Many ELISA assays, including antibody titer determination and isotyping, can be implemented readily on the GatorPrime system.

| Gator probe | Function | Application | Dynamic range | Regeneration |
|---|--|-----------------|-------------------|--------------|
| Protein A | Binds IgG's of various species including human and mouse | Q/K/QKR | 0.02 – 2000 µg/mL | Yes |
| Protein G | Binds IgG's of various species including human and rat | Q/K/QKR | 0.02 – 2000 µg/mL | Yes |
| Protein L | Binds IgG's of various species through the kappa light chain | Q/K/QKR | 0.1 – 2000 µg/mL | Yes |
| Human Fc | Immobilize human Fc-fusion protein or human IgG for quantitative or kinetic analysis | Q/K/EP | 0.02 – 300 µg/mL | Yes |
| Mouse Fc | Immobilize mouse Fc-fusion protein or mouse IgG for quantitative or kinetic analysis | Q/K/EP | 0.02 – 300 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 |
| Streptavidin (SA) | Binds biotinylated and Avi-tagged biomolecules | Indirect Q/K/EP | Protein-dependent | No |
| Small Molecule, Antibody, and Protein (SMAP) | Binds biotinylated and Avi-tagged biomolecules and subsequent binding of small molecules and protein | Indirect Q/K | >150 Da | No |
| APS (Aminopropylsilane) | Binds hydrophobic proteins | Q | Protein-dependent | No |
| Amine-Reactive | Covalently attach amine group of proteins using EDC/NHS | Indirect Q/K/EP | Protein-dependent | No |
| Custom-Made | Custom made biosensors for your specific applications (Ex: SARS-CoV-2 R&D biosensors for COVID research) | Varies | Varies | Varies |

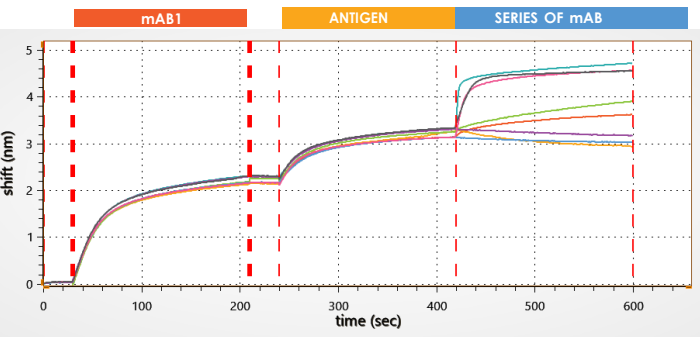
Epitope binning with GatorPrime system

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

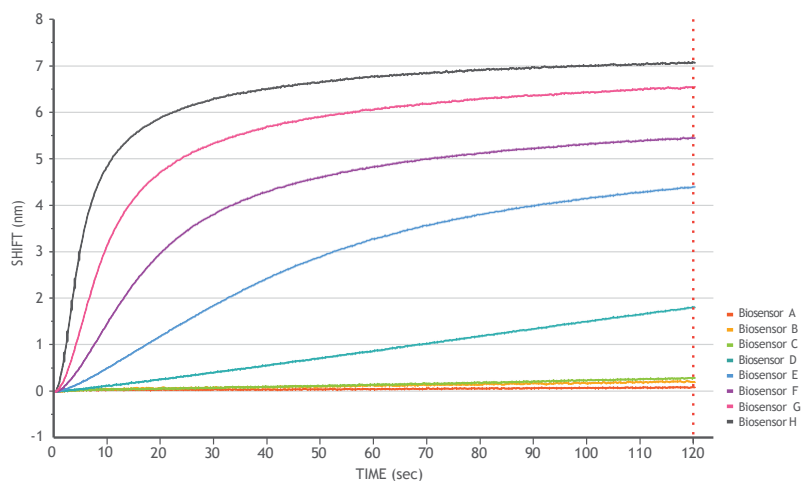
| | mAb1 | | |
|-------|--|-------|--|
| mAb1 | Not binding to antigen when mAb1 is bound; same epitope | mAb1 | Binding to antigen when mAb1 is bound; different epitope |
| mAb2 | Not binding to antigen when mAb1 is bound; same epitope | mAb2 | Binding to antigen when mAb1 is bound; different epitope |
| mAb3 | Not binding to antigen when mAb1 is bound; same epitope | mAb3 | Binding to antigen when mAb1 is bound; different epitope |
| mAb4 | Ambiguous results | mAb5 | Binding to antigen when mAb1 is bound; different epitope |
| mAb5 | Not binding to antigen when mAb1 is bound; same epitope | mAb7 | Binding to antigen when mAb1 is bound; different epitope |
| mAb6 | Binding to antigen when mAb1 is bound; different epitope | mAb8 | Binding to antigen when mAb1 is bound; different epitope |
| mAb7 | Not binding to antigen when mAb1 is bound; same epitope | mAb9 | Binding to antigen when mAb1 is bound; different epitope |
| mAb8 | Not binding to antigen when mAb1 is bound; same epitope | mAb11 | Binding to antigen when mAb1 is bound; different epitope |
| mAb9 | Not binding to antigen when mAb1 is bound; same epitope | mAb12 | Binding to antigen when mAb1 is bound; different epitope |
| mAb10 | Binding to antigen when mAb1 is bound; different epitope | | |
| mAb11 | Not binding to antigen when mAb1 is bound; same epitope | | |
| mAb12 | Not binding to antigen when mAb1 is bound; same epitope | | |
| mAb14 | Binding to antigen when mAb1 is bound; different epitope | | |
| mAb15 | Binding to antigen when mAb1 is bound; different epitope | | |
| mAb16 | Binding to antigen when mAb1 is bound; different epitope | | |

The results shown here suggest two different epitope groups.

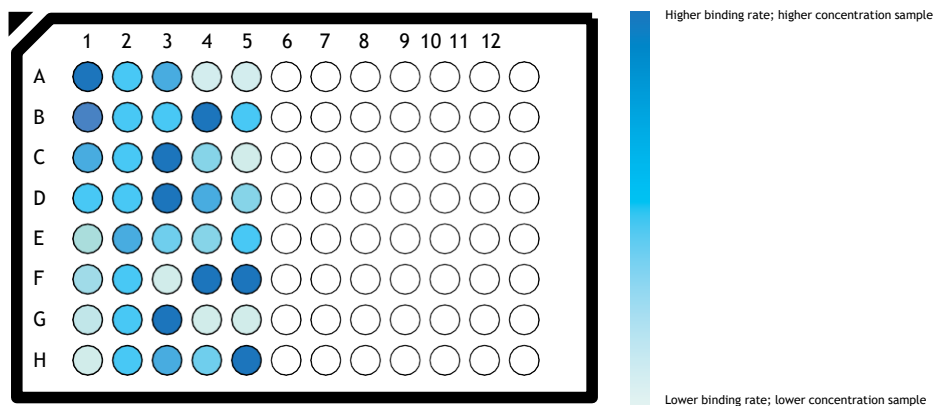
Antibody titer using Gator Bio Protein A biosensors

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



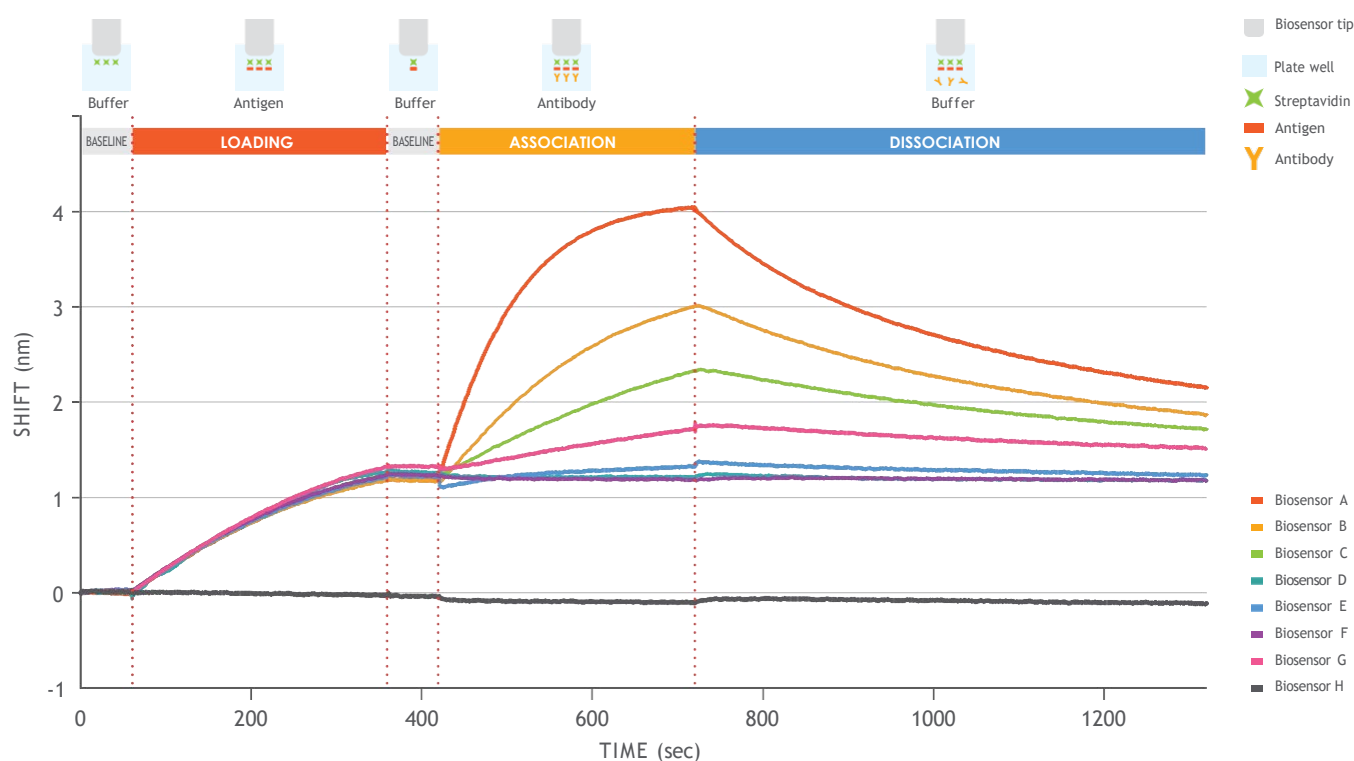
Heat map generated by GatorPrime software for human IgG concentration analysis using Gator Bio Protein A biosensors



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



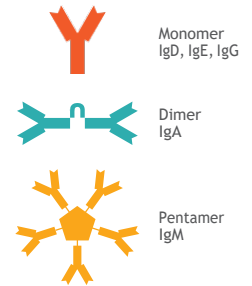
Highlights

- 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

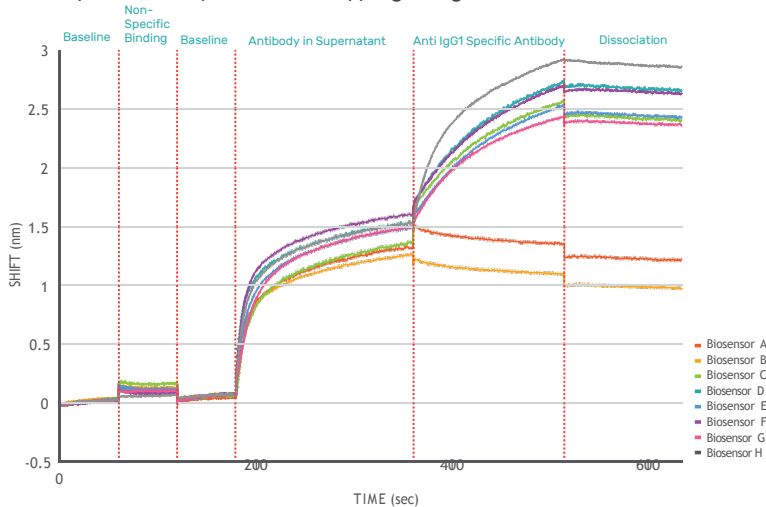
Antibody isotyping and subtyping with GatorPrime system

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 presence of 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

| 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 |
| Power | 200W |

| SOFTWARE |
|--|
| <ul style="list-style-type: none"> • 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, DNA, RNA, liposomes, cells, viruses, etc. |
| Work flow | <ul style="list-style-type: none"> • 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 | <ul style="list-style-type: none"> • Yes or no binding • Relative and absolute concentration • Kinetics and affinity |

| QUANTITATION | |
|---|--|
| Analysis time | <ul style="list-style-type: none"> • IgG concentration in 30 seconds for 8 samples • <10 minutes for 96 samples |
| Linear range for Gator Protein A biosensor | 0.025 – 2,000 µg/mL |

| KINETICS | |
|---|--|
| Analysis time | Real-time kinetic binding from 1 minute to 4 hours |
| Association rate k_{on} | $10^1 - 10^6 \text{ M}^{-1}/\text{s}$ |
| Dissociation rate k_{off} | $10^{-6} - 10^{-1}/\text{s}$ |
| Affinity constant K_D | 10 pM – 1 mM |
| Binding curve baseline noise | <3 pm (RMS) |
| Binding curve baseline drift | <40 pm/hour (RMS) |