Application Note

5-log dynamic range sensitivity for mouse hybridoma screening using the GatorPlus™ system with anti-mouse Fc biosensor

Indrani Chakraborty, Daniel Egen, Kathryn Livingston, Shannon Moore, Rich Wang, Chip Slaybaugh

Gator Bio, Palo Alto, CA

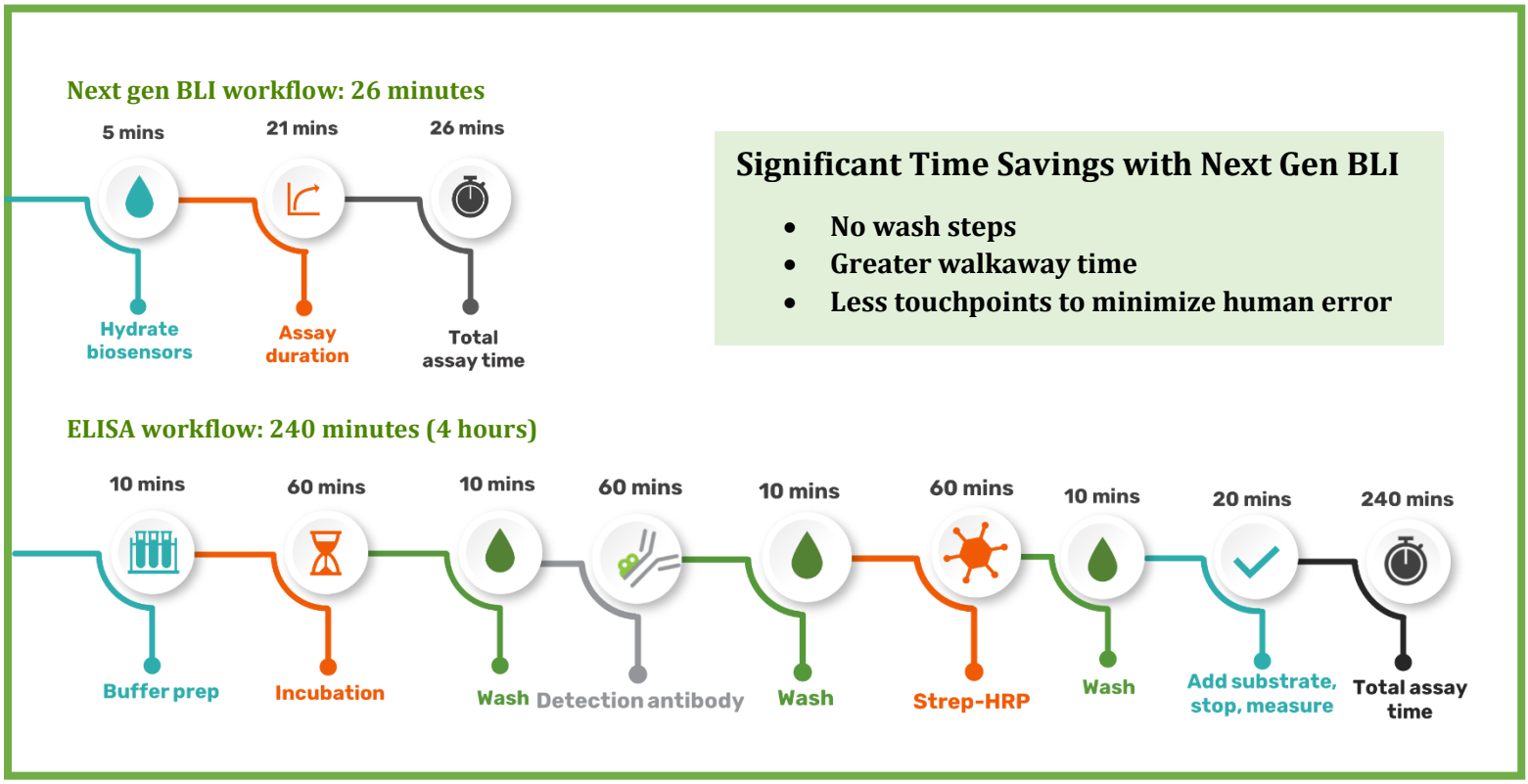
Introduction

Antibodies in supernatants from hybridomas are commonly selected by enzyme-linked immunosorbent assays (ELISA) through a screening process based on a combination of antibody concentration and affinity. However, hybridomas that express low levels of a high-affinity antibody can be missed during ELISA's washing steps. Traditional BLI eliminates the need for washing steps, making it a popular substitute to ELISA for antibody screening. However, traditional BLI has a limitation on sensitivity and dynamic range. Next gen BLI enables rapid antibody screening with a wide dynamic range and increased sensitivity. This application note describes the use of the GatorPlus system and anti-mouse Fc (MFC) biosensors for rapid and efficient hybridoma screening.

Easy quantitation of mouse IgGs

MFC biosensors are built using proprietary fiber optic technology to provide better sensitivity, wider dynamic range, and 20x regeneration capability for antibody quantitation assays. The branched polysaccharide design provides multiple binding sites for a significant improvement in binding capacity.

GatorPlus with MFC biosensor eliminates the labor-intensive wash steps required in ELISA workflows. The Gator™ platform simplifies the antibody screening workflow and can provide a time savings of 89% vs. ELISA.



Materials required

- GatorPlus instrument with GatorOne software
- MFC biosensors (Gator Bio PN 160004)
- Max plate (Gator Bio 130018)
- For all Gator instruments: 96-well , black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 655209)
- Optional for GatorPlus instrument: 384-well, black (Greiner 781900)
- 1x PBS (Sigma P3813-10PK)
- mIgG (Equitech-Bio SLM56-1000)
- Q buffer (Gator Bio PN 120019)

General Assay Settings

	High Concentration	Low Concentration
Concentration	1 – 2000 µg/mL	0.02 – 5 µg/mL
Assay time	30 seconds	5 minutes
RPM	400 rpm	1000 rpm

Table 1: General assay settings for high and low concentration ranges including instrument settings for proper standard curve

Standard Curve Preparation and Result

The quantitation assay is calibrated by using a pure antibody of known concentration. Since GatorPlus can quantify a wide concentration range of mouse Fc samples, a standard curve in the appropriate concentration range is generated first. The two concentration ranges require different instrument settings as noted in Table 1.

Preparation

Using the standard probe hydration protocols, hydrate the biosensors with assay buffer for a minimum of 5 minutes prior to use.

If the antibody standard is supplied lyophilized, then it must be reconstituted prior to first use. To reconstitute the standard:

1. Spin the tube in a table-top centrifuge to collect all powder on the bottom
2. Add the appropriate volume of standard diluent (the same diluent should be used for dilution of any samples)
3. Mix and let sit for 5 minutes at room temperature and mix again and aliquot the stock sample into single use vials
4. Freeze remaining standard solution at -20°C or below

Serial dilutions of standard

A standard curve is prepared by making serial dilutions of the IgG standard within a range of concentrations near the expected concentrations of the unknown samples. Examples of a dilution series for a standard curve ranging from 0 – 2000 µg/ml (Table 2) and 0 – 5 µg/ml (Table 3) are below.

Stock = 10 mg/ml

	ml/stock	Q buffer	Final concentration
A	60 µL	240 µL	2000 µg/ml
B	100 µL (A)	200 µL	666.67 µg/ml
C	100 µL (B)	200 µL	222.22 µg/ml
D	100 µL (C)	200 µL	74.07 µg/ml
E	100 µL (D)	200 µL	24.69 µg/ml
F	100 µL (E)	200 µL	8.23 µg/ml
G	100 µL (F)	200 µL	2.74 µg/ml
H	0 µL	200 µL	0 µg/ml

Table 2: Dilution series for standard curve from 0-2000 µg/ml

Stock = 100 µg/ml

	ml/stock	Q buffer	Final concentration
A	15 µL	285 µL	5 µg/ml
B	100 µL (A)	200 µL	1.6 µg/ml
C	100 µL (B)	200 µL	0.55 µg/ml
D	100 µL (C)	200 µL	0.18 µg/ml
E	100 µL (D)	200 µL	0.06 µg/ml
F	100 µL (E)	200 µL	0.02 µg/ml
G	100 µL (F)	200 µL	0.006 µg/ml
H	0 µL	200 µL	0 µg/ml

Table 3: Dilution series for standard curve from 0-5 µg/ml

Assay setup and data analysis

1. Open Gator One software and choose “Q” from “Quick start”, rename experiment, and enter assay description and user information.

2. Under “Basic Parameters”, select “96 well plate” and “tilt”. The Equilibration Settings are 300 sec (high concentration) or 600 sec (low concentration), and set up shaking speed (rpm) for both shaker A and shaker B (Table 1).

3. Plate set up indicates where the standards/samples are in the 96 Plate (Shaker A) and Max Plate (Shaker B). To define a column of wells, click the number above the column and click the button corresponding to the well identity (e.g., Standard Unknown, Regeneration).

4. In “Assay Steps”, assign reaction time (30 sec) and shaking speed (Table 1); regeneration time (5 sec) and speed (1000 rpm).

Note: “Regeneration Before Assay” setting can be turned on or off. To ensure reproducibility, regeneration prior to initial use of probe is not recommended.

5. Under the “Preview” tab, toggle through the assay to verify the steps. Save the assay template for future use, then click “Start”.

6. Once the assay is completed, select the “New Q Analysis” tab and “Binding Curve Fit” to populate the table with binding rates. Calculate the concentrations with “Calculate Conc”. The binding model can be selected under “Parameters”, but the default setting is recommended.

7. In the Report section, select included factors and export report in selected file format.

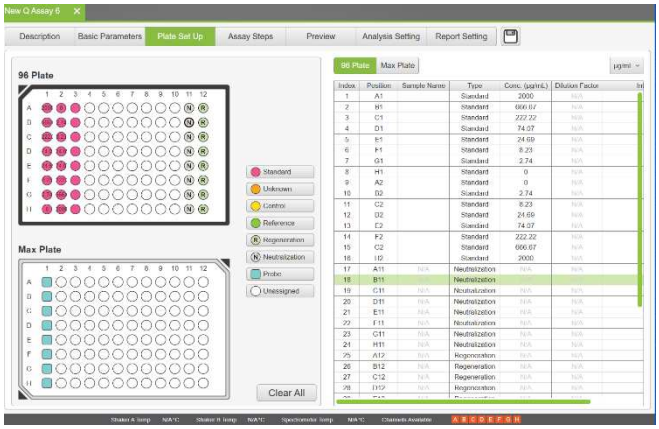


Figure 1: Plate set up.

Result of high concentration standard curve

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 4: Calculated average concentrations and CV% ranging from 2.74 – 2000 µg/ml. This is the average of 3 sets of 8 MFC biosensors.

Result of low concentration standard curve

Known concentration (µg/ml)	Avg. calculated concentration (µg/ml)	CV (%)
5	4.967	1.3
1.667	1.780	5.4
0.556	0.520	4.4
0.185	0.187	3.0
0.062	0.065	1.3
0.021	0.021	0.0

Table 5: Calculated average concentrations and CV% ranging from 0.021 – 5 µg/ml. This is the average of 3 sets of 8 MFC biosensors.

Conclusion

The Gator™ platform is the next generation of biolayer interferometry. In addition to the benefits that traditional BLI offers, the novel proprietary fiber optic technology provides better sensitivity, wider dynamic range, and 20x regeneration capability.

The methods for obtaining 5 log dynamic range sensitivity detection with GatorPlus have been outlined in this application note alongside recommendations for assay setup and data analysis.

Hybridoma screening on GatorPlus is extremely useful for evaluating low concentration hybridoma samples. With new surface chemistry and larger surface area, only 30 seconds are required for high concentration sample analysis. Together with easy to use software, the Gator platform is ideal for high quality, reproducible and cost-effective screening of hybridoma supernatant.

References

Latesh Lad et al., High-Throughput Kinetic Screening of Hybridomas to Identify High-Affinity Antibodies Using Bio-Layer Interferometry. Journal of Biomolecular Screening 2015, Vol.20(4) 498-507

Anti-Murine IgG (AMQ) Biosensors-for Determination of Antibody Concentration. Product Bulletin. https://www.sartorius.com/download/552174/7/anti-murine-igg-biosensors-daatasheet-en-sartorius-data.pdf

Mouse Fc (MFC) Probes. Product Bulletin. Gator_Product_Insert_MFC.pdf (gatorbio.com)

Ordering information

To request a quote, visit www.gatorbio.com/contact or email sales@gatorbio.com

Gator Bio, Inc.
2454 Embarcadero Way, Palo Alto, CA
+1 855 208 0743 • GatorBio.com