APPLICATION NOTE

5-log dynamic range sensitivity for mouse hybridoma screening using the Gator® Plus system with Anti-Mouse IgG Fc (MFC) Probes

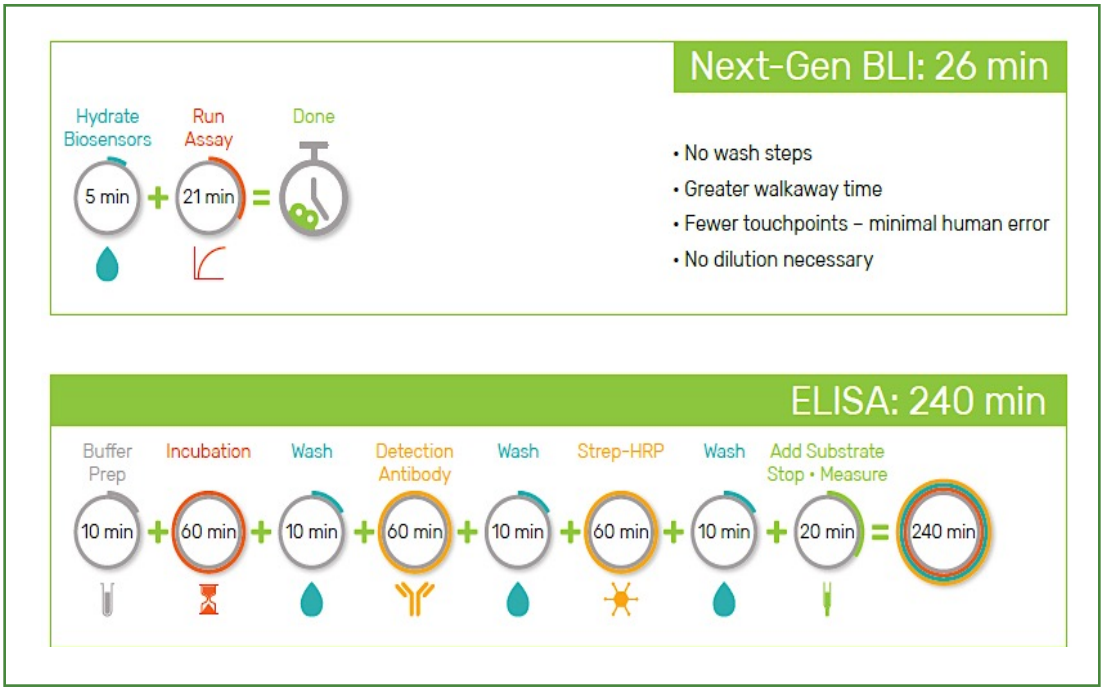
INTRODUCTION

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.[1] However, hybridomas that express low levels of a high-affinity antibody can be missed during ELISA's washing steps. Traditional Bio-Layer Interferometry (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 Gator® Plus system and Anti-Mouse IgG Fc (MFC) Probes for rapid and efficient hybridoma screening.

EASY QUANTIFICATION OF MOUSE IGGS

Gator® MFC probes 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.

Gator® Plus with MFC probes eliminate 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.



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MATERIALS REQUIRED

- MFC Probes: PN 160004
- Max Plate: PN 130018
- Black Plate 96-well: Greiner 655209
  384-well: Greiner 781209
- 1x Phosphate Buffered Saline (PBS):
  Sigma P3813-10PK
- Mouse IgG (mIgG): Equitech-Bio SLM56-1000

GENERAL ASSAY SETTINGS

	High Concentration	Low Concentration
Conc (µg/mL)	1–2000	0.02–5
Assay time (sec)	30	300
RPM	400	1000

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 Gator® Plus can quantify a wide concentration range of mouse IgG (mIgG) 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 probes 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 standards:

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. Mix again and aliquot the stock sample into single use vials
4. Freeze the remaining standard solution at -20°C - 80°C

SERIAL DILUTIONS OF STANDARD

A standard curve is prepared by making serial dilutions of the mouse IgG standards 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. A 10 mg/mL mouse IgG stock in the same diluent is prepared in Table 2 and a 100 ug/mL mouse IgG stock in the same diluent is prepared in Table 3 in advance.

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

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

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

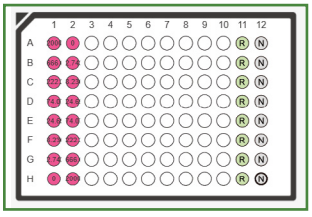
Table 3: Dilution series for standard curve from 0 - 5 µg/mL

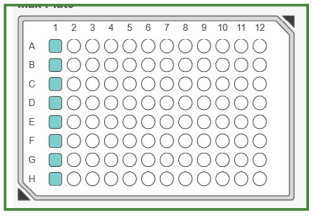
ASSAY SETUP AND DATA ANALYSIS

1. Open GatorOne Software and select "Q" from "Quick Start". Rename the experiment, and enter the assay description and the 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 specify the shaking speed (rpm) for both Shaker A and Shaker B (Table 1).
3. Plate set up indicates the locations of the standards and samples in the 96-well plate (Shaker A) and Max Plate (Shaker B). To define a column of wells, select both the column number and the tab corresponding to the well identity (e.g., Standard, Unknown, Regeneration).
4. Under "Assay Steps", assign the reaction time (30 sec) and the shaking speed (Table 1), followed by the 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 the initial use of the probe is recommended.

5. Under the "Preview" tab, verify the steps of the assay, and click "Save". 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 the included factors and export the report in selected file format.





Index	Position	Sample Name	Type	Conc. (µg/mL)	Dilution Factor
1	A1	mIgG	Standard	2.00E+003	N/A
2	B1	mIgG	Standard	666.667	N/A
3	C1	mIgG	Standard	222.222	N/A
4	D1	mIgG	Standard	74.074	N/A
5	E1	mIgG	Standard	24.691	N/A
6	F1	mIgG	Standard	8.23	N/A
7	G1	mIgG	Standard	2.743	N/A
8	H1	mIgG	Standard	0	N/A
9	A2	mIgG	Standard	0	N/A
10	B2	mIgG	Standard	2.743	N/A
11	C2	mIgG	Standard	8.23	N/A
12	D2	mIgG	Standard	24.691	N/A
13	E2	mIgG	Standard	74.074	N/A
14	F2	mIgG	Standard	222.222	N/A
15	G2	mIgG	Standard	666.667	N/A
16	H2	mIgG	Standard	2.00E+003	N/A

Figure 1: Plate set up

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RESULT OF HIGH CONCENTRATION STANDARD CURVE

Known Concentration (µg/ml)	Average Calculated Concentration (µg/ml)	% CV (n=3)
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: Average calculated concentration and % CV of triplicates of high concentration standard curve ranging from 2.7-2000 µg/mL

RESULT OF LOW CONCENTRATION STANDARD CURVE

Known Concentration (µg/ml)	Average Calculated Concentration (µg/ml)	% CV (n=3)
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: Average calculated concentration and % CV of triplicates of low concentration standard curve ranging from 0.021-5 µg/mL

CONCLUSION

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

The method for obtaining a 5-log dynamic range sensitivity detection with the Gator® Plus have been outlined in this application note alongside recommendations for assay setup and data analysis.

Hybridoma screening on the Gator® Plus is extremely useful for evaluating hybridoma samples at low concentrations. With the 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 supernatants.

REFERENCES

1. 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

ORDER ONLINE BELOW:

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