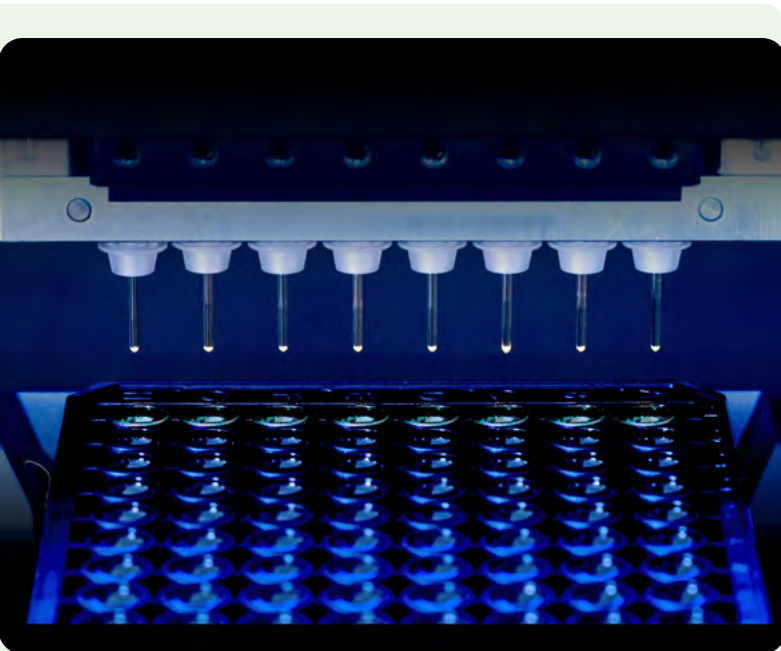


MFC XT BIOSENSOR FOR HYBRIDOMA SCREENING

Single-Assay Hybridoma for Expression and Affinity of Mouse IgGs

ABSTRACT

Hybridoma panels remain a cornerstone of therapeutic and research antibody generation. Hybridoma screening requires the rapid assessment of hundreds to thousands of clones for secretion, titer, and binding characteristics. At this scale, traditional ELISA-based workflows are stifled by significant manual burden and provide no straightforward path to early exclusion of poor binders. This application note demonstrates how Gator® Anti-Mouse IgG Fc (MFC) XT Biosensors enable quantitation, off-rate ranking, and affinity-based candidate triage in a single automated Biolayer Interferometry (BLI) run. Compared to typical BLI biosensors, MFC XT Biosensors offer superior per-molecule signal resolution, a broad dynamic range of 0.02–10,000 µg/mL, and robust regeneration for up to 20 cycles. Practical guidelines for implementing this workflow, including candidate ranking strategies and clone exclusion criteria, are covered in detail.



INTRODUCTION

The hybridoma screening challenge

Although artificial intelligence and display library screening have taken much of the spotlight for their speed, they lack many advantages of mouse hybridoma screening methodology, such as:

1. Antibodies derived from hybridomas undergo *in vivo* affinity maturation, producing candidates with refined selectivity profiles that naïve display library methods typically cannot replicate without additional directed evolution steps.
2. Hybridoma-derived antibodies are generated through natural B cell development, meaning they tend to exhibit favorable biophysical properties such as reduced propensity for aggregation and greater thermal stability compared to many display library-derived candidates.
3. *In vivo* immunization with intact or membrane-embedded antigens enables the generation of antibodies against difficult target classes
 - including GPCRs, ion channels, and heavily glycosylated proteins that remain challenging for both computational design and display library approaches.

Following the early steps of cell fusion and HAT isolation, successful campaigns routinely yield hundreds or thousands of clones, none of which are purified at the screening stage. The most common approach to hybridoma screening at this scale is to use ELISA, which is scalable, but only provides endpoint data, and is prone to poor intra- and inter-assay reproducibility (%CV). Biolayer Interferometry (BLI) addresses these limitations by delivering reproducible real-time interaction data directly from crude hybridoma supernatants without requiring sample purification. BLI is commonly used in hybridoma screening for:

- Measuring IgG secretion and approximate titer
- Ranking candidates based on dissociation rate (k_{off})
- Cross-reactivity and epitope group assignment

HOW BLI WORKS

BLI measures changes in the interference pattern of white light reflected from the surface of a biosensor probe. As molecules bind to or dissociate from the probe tip, the optical path length changes, producing a measurable shift in the interference spectrum (measured as nm shift). Since the signal depends only on molecules at the probe surface, BLI is highly tolerant of matrix effects from crude cell culture media when proper experimental steps are taken to limit these effects (Appendix 2).

MATERIALS AND METHODS

Reagents and equipment

Item	Origin	Specification / Part Number
Gator® Plus	Gator Bio	PN 110022
BLI 384-Tilt Plate, Polypropylene	Gator Bio	PN 130161
MFC XT Probes	Gator Bio	PN 160049
Q Buffer	Gator Bio	PN 120008
Regen buffer (No salt)	Gator Bio	PN 120063
Mouse IgG standard	User supplied	
Target antigen	User supplied	
Hybridoma supernatants	User supplied	

Biosensor selection

The Gator MFC XT Probe is a next-generation anti-mouse IgG Fc capture biosensor engineered to outperform first-generation Mouse Fc biosensors in hybridoma workflows and other kinetics applications. Key design improvements over first-generation MFC biosensors include:

- Greater signal-per-molecule using Gator's proprietary XT optical coating
- Broader dynamic range enables quantitation without dramatic pre-dilution across the range of typical hybridoma titers
- Monomeric capture molecule, reducing incidence of avidity for kinetics applications
- High subclass coverage: IgG1, IgG2a, IgG2b, and IgG3
- High specificity for mouse IgG with low cross-reactivity to other IgG species
- Robust regeneration chemistry supporting up to 20 cycles per probe

Assay workflow overview

The MFC XT hybridoma screening workflow is structured in a five-step process enabling first-pass quantitation and off-rate ranking with a single re-usable biosensor.

Step 1 – Biosensor equilibration and baseline

- Pre-wet MFC XT biosensors in Q buffer for 10 minutes
- Instrument transfers biosensors to Q buffer well for 60 seconds to measure baseline prior to sample loading

Step 2 – Mouse IgG capture and quantitation

- Instrument transfers biosensors into hybridoma supernatant wells (diluted 1:1 in Q buffer to reduce matrix effects and signal deviations while maintaining detectable signal)
- Association time: 300 seconds (adjust depending on expected titer)

Step 3 – Second Baseline

- Instrument transfers IgG-loaded biosensors to buffer blank well
- Second Baseline time: 60 seconds

Note: if second baseline loses more than 20% of its signal after loading, it is likely to be non-specific binding from the matrix dissociating from the sensor. Consider increasing dilution or extending second baseline to ensure a flatter baseline for the association/dissociation steps.

Step 4 – Antigen association

- Instrument transfers IgG-loaded biosensors to wells containing a single concentration of antigen (diluted in Q buffer)
- Antigen concentration: 500 nM
- Association time: 180 seconds

Note: At least one ligand well (step 2) should be a blank or similar negative control to detect non-specific signal as a result of direct antigen binding to the biosensor or refractive index changes from solvents in the antigen wells (e.g. DMSO, Glycerol, Sucrose, etc.)

Note: Antigen concentration assumes a naïve panel. For panels with a stronger expected affinity, a lower concentration can be used to better discriminate candidates.

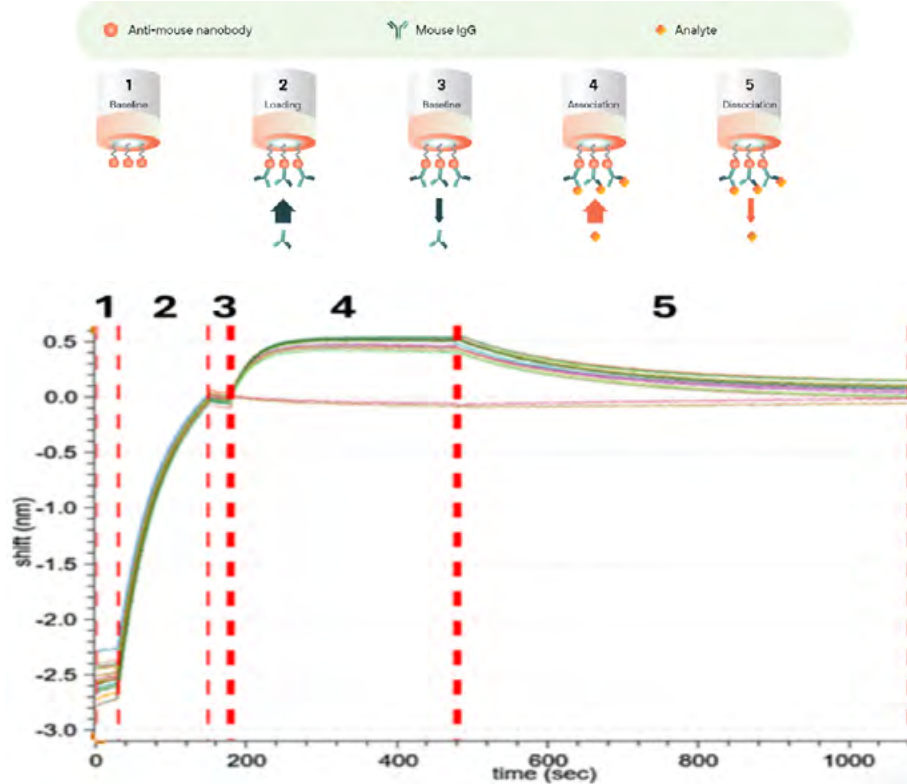


Figure 1: Five-step BLI hybridoma screening workflow: 1) probe equilibration and baseline, 2) mouse IgG capture from crude supernatant, 3) second baseline, 4) antigen association, 5) dissociation.

Step 5 – Antigen dissociation

- Instrument transfers biosensors to a well containing only Q buffer
- Dissociation: 180+ seconds in Q buffer to characterize k_{off} (dissociation rate)

Note: Adjust time depending on quality of the panel – early panels can be screened in 180 seconds (faster k_{off}); panels that have undergone affinity maturation may need 600 seconds or more.

Biosensor regeneration

- Instrument transfers biosensors between Regen Buffer (No Salt) and Q buffer 3-5 times in 5-second pulses
- Biosensors can typically be reused for up to 20 cycles with <15% signal loss (see Appendix 1)

Note: Optimization of regeneration conditions should be performed when working with unusual supernatant conditions or antibody constructs

Identifying concentration from screening data

- Using GatorOne kinetics analysis software under 3: Set Reference, select the loading step on the graph and use "Quantitate" to enable quantitation mode.
- Signal (nm) read against previously-measured 7-point mouse IgG standard curve (0.02-1.28 $\mu\text{g/mL}$) with blank subtracted.

Candidate ranking

Exclusion criteria

When association responses are weak or absent, kinetics fitting software may return artifactually low k_{off} values, since dissociation cannot be meaningfully measured in the absence of a real binding signal. Exclusion criteria prevent these false positives from advancing.

- Clones with loading response below 0.2 nm (< 0.20 $\mu\text{g/mL}$ final concentration) or with atypical loading curves can be flagged as non-secretors (or IgM secretors if they still show antigen binding).

Note: This cutoff is empirically derived and specific to the MFC XT biosensor and mouse antibody panels — individual adaptation may be required.

- Samples with bound Ab showing poor antigen binding are classified as non-binders and excluded. Many users classify poor binders based on absolute antigen binding response, and this is a valid approach, but it ignores variability in antibody loading across clones. In this case, individual clone antigen:load ratios are compared to the panel's average ratio - candidates whose ratio is ½ the panel's average are excluded as poor binders.

Note: All exclusion criteria are arbitrary. This method simply gives a rational approach for comparing binding efficacy across different hybridomas. This cutoff can be adjusted to be more or less stringent depending on the nature of the panel.

Candidate ranking

- Single-concentration kinetics results are fitted with GatorOne software using a 1:1 model.
- Remaining binders ranked by k_{off} (dissociation rate) for rapid lead prioritization.

Lead clones identified by off-rate ranking proceed to a full kinetics assay in a follow-up experiment. While users often perform screening experiments with unpurified antibodies, a multi-concentration kinetics assay is typically performed using purified antibodies. Selected antibodies are exposed to a 5-7 point serial dilution of antigen (typically starting 20x above the estimated K_D from screening and diluting twofold per point) alongside controls (e.g. zero antigen blank). Kinetics values (k_{on} , k_{off} and K_D) are calculated by global 1:1 Langmuir fitting in GatorOne software.

RESULTS AND DISCUSSION

Quantitation of Mouse IgG on the MFC XT Biosensor

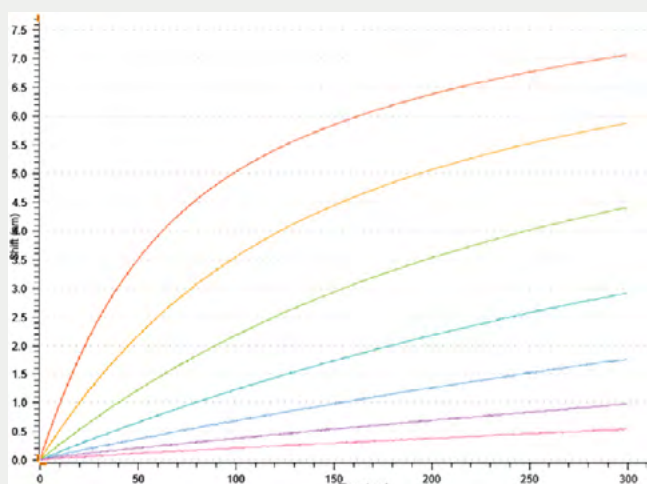
96 antibodies from the hybridoma panel were compared to a standard curve of mouse IgG for concentration determination. The standard curve is provided below in Figure 2.

Note: The Gator MFC XT biosensor can be used to detect a higher range of antibodies for quantitation. To obtain higher-titer results up to 10,000 $\mu\text{g/ml}$, an experiment at 25°C and at 400 RPM for 10-30 seconds is appropriate.

A

Assay parameters	Known Conc	Calculated Conc	Initial Binding Rate	%Recovery
	$\mu\text{g/ml}$	$\mu\text{g/ml}$	(nm/sec)	
300 Sec 1000 RPM	1.28	1.294	0.106	101.1%
	0.64	0.608	0.0497	94.9%
	0.32	0.326	0.0265	101.9%
	0.16	0.166	0.0133	103.5%
	0.08	0.085	0.0067	106.1%
	0.04	0.040	0.0030	99.4%
	0.02	0.023	0.0016	112.6%

B



C

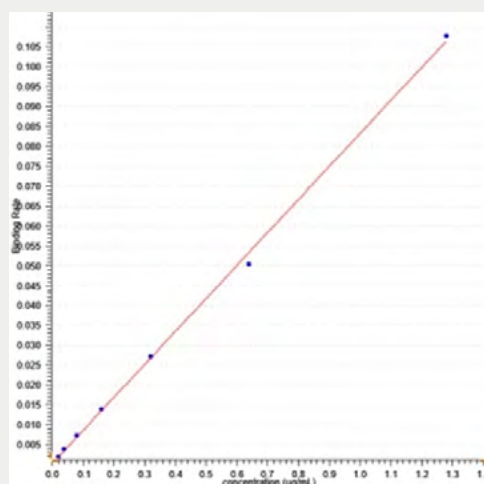


Figure 2: Quantitation standard curve.

A: Assay parameters and results of standard curve for mouse IgG.

B: Appearance of raw sensorgrams at the indicated assay parameters and concentrations.

C: Standard curve output using initial binding rate (nm/sec) using a linear regression for fitting.



CANDIDATE RANKING AND EXCLUSION

A selection of 48 out of 96 tested samples is shown in Figure 3 and identified with a grid system. Of these 48, the following were excluded based on selection criteria outlined in methods section:

- **Insufficient loading response (Loading step not shown)**
 - Cutoff set to 0.2 nm load (see methods)
 - Newly excluded: A4, B5, B6, C5, C6, E3, F1, G4, H2, H4
 - Note: kinetics software returned an artifactually low k_{off} value for E3 and H4. This is an example of how filters can help weed out deceptive fitting results.
- **Poor response-to-load**
 - The average antigen binding response was 76% of antibody loading response. Load:Antigen ratios lower than 38% (1/2 of panel average of 76%) are classified as low/poor binders (or IgM secretors)
 - Newly excluded: A6, B2

Candidates that were not eliminated based on these criteria were ranked on k_{off} values. The top five candidates from this selection of 48 are given below.

Note: k_{off} values lower than $1.00 \times 10^{-6} \text{ s}^{-1}$ are typically considered out-of-range for BLI. However, none of these values should be considered definitive at this stage – they should only be used for ranking candidates at this stage, given that the dissociation time is only 180 seconds.

- | | |
|------|--|
| • D6 | $k_{off}: <1.00 \times 10^{-6} \text{ s}^{-1}$ |
| • G2 | $k_{off}: <1.00 \times 10^{-6} \text{ s}^{-1}$ |
| • D2 | $k_{off}: 6.20 \times 10^{-5} \text{ s}^{-1}$ |
| • D1 | $k_{off}: 1.32 \times 10^{-4} \text{ s}^{-1}$ |
| • E2 | $k_{off}: 1.77 \times 10^{-4} \text{ s}^{-1}$ |

These five candidates, selected purely on the basis of off-rate ranking from crude supernatant, represent the output of a complete first-pass screen and are well-positioned for definitive K_D determination in a multi-concentration kinetics assay.

Note: Accurate kinetics values cannot be obtained with a single data point, particularly one at 500 nM, since the K_D for these top five candidates is likely 1 nM or below based on the k_{off} rates shown. As such, a good high-concentration starting point for the titration series used in the kinetics assay would be 20–50x above the predicted K_D from these experiments.

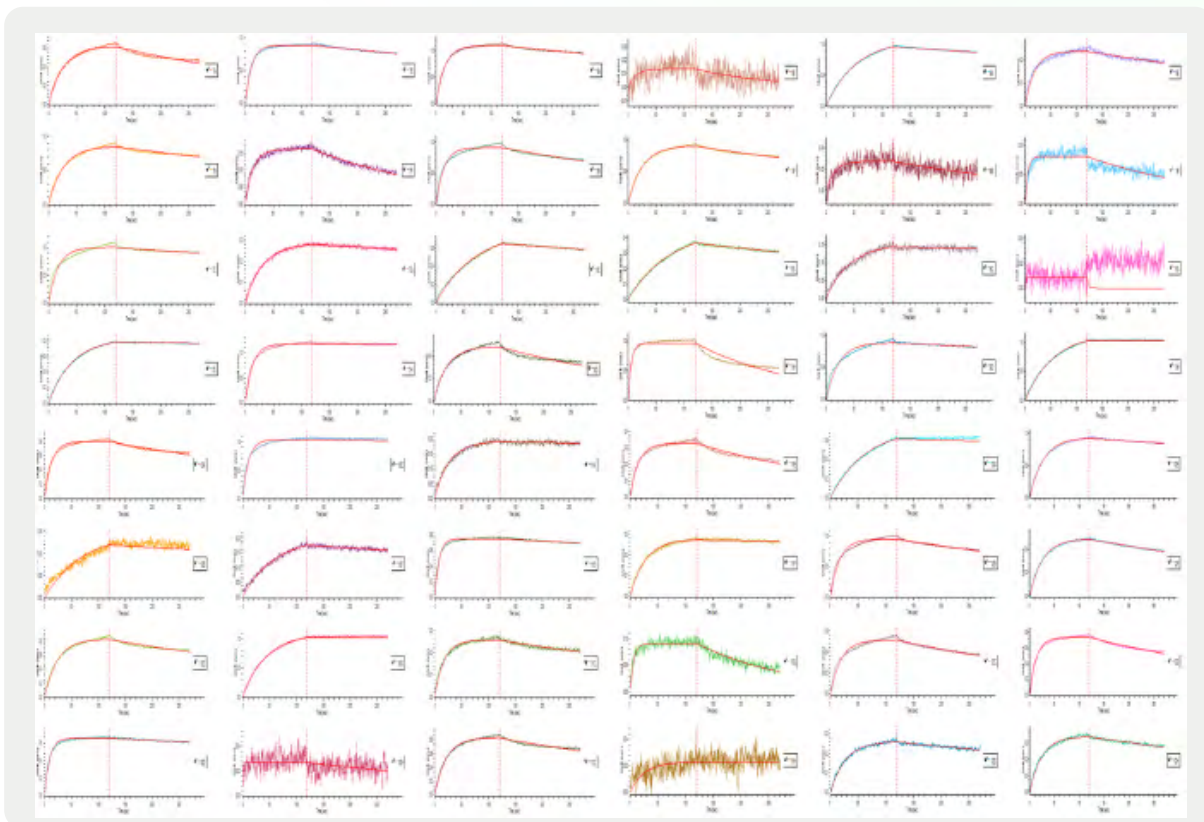


Figure 3: 48 representative traces from a 96-sample hybridoma screen presented in grid format for reference. Colored traces indicate binders; excluded clones are identified by grid position.

CONCLUSION

Because hybridoma screening practices vary widely between laboratories and many workflows remain unpublished, reproducible and well-documented protocols are particularly valuable in this field. Applying the techniques covered in this application note enables users to obtain consistent, high-quality results when performing mouse hybridoma screening.

Moreover, as demonstrated herein, MFC XT Biosensors address the primary bottlenecks in hybridoma screening workflows:

- Sensitivity at low titers alongside rapid loading response improving throughput for large clone sets.
- Expanded dynamic range (0.02–10,000 µg/mL) eliminates the pre-dilution step required with first-generation biosensors for high-titer clones, a common source of quantitation error and workflow interruption.
- Stable performance through multiple regeneration cycles (Appendix 1) as well as strong performance across a variety of media conditions (Appendix 2), making them an easy-to-deploy tool for any hybridoma laboratory and significantly reducing per-assay cost for high-throughput antibody characterization workflows.

ADDITIONAL RESOURCES

Lad et al. "High-Throughput Kinetic Screening of Hybridomas to Identify High-Affinity Antibodies Using Bio-Layer Interferometry," SLAS Discovery, 20(4): 498-507, 2015.

Säfsten P, Klakamp SL, Drake AW, Karlsson R, Myska DG, "Screening antibody-antigen interactions in parallel using Biacore A100," Analytical Biochemistry, 353(2):181-190, 2006.

Ylera et al. "Off-Rate Screening for Selection of High-Affinity Anti-Drug Antibodies," Analytical Biochemistry, 441(2), 2013.

Concentration µg/mL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
7.8	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
15.6	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
31.3	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
62.5	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.23	0.24	0.23	0.24	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
125	0.47	0.47	0.48	0.47	0.47	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.45	0.45	0.45	0.45	0.45	0.45	0.45
250	0.88	0.88	0.88	0.88	0.87	0.87	0.86	0.86	0.85	0.85	0.85	0.84	0.84	0.84	0.83	0.83	0.83	0.82	0.82	0.82
500	1.49	1.49	1.50	1.48	1.47	1.46	1.47	1.45	1.46	1.44	1.43	1.43	1.43	1.42	1.43	1.42	1.41	1.41	1.41	1.38
1000	2.59	2.56	2.57	2.53	2.50	2.51	2.47	2.47	2.48	2.49	2.44	2.45	2.42	2.39	2.44	2.40	2.39	2.40	2.34	2.40

Binding Rate change % by regeneration cycle																				
7.8	0%	2%	0%	-2%	-2%	-3%	-1%	-1%	-2%	-4%	-4%	-5%	-3%	-6%	-4%	-5%	-5%	-5%	-5%	-7%
15.6	0%	0%	-1%	0%	0%	-1%	0%	-1%	-1%	-3%	-3%	-4%	-5%	-4%	-4%	-5%	-4%	-6%	-5%	-6%
31.3	0%	1%	1%	0%	-1%	-1%	-1%	-2%	-3%	-3%	-3%	-4%	-4%	-4%	-2%	-3%	-3%	-5%	-4%	-6%
62.5	0%	0%	0%	-1%	-1%	-1%	-1%	-1%	-3%	-2%	-3%	-2%	-3%	-4%	-4%	-4%	-4%	-5%	-5%	-5%
125	0%	-1%	0%	-1%	-1%	-2%	-3%	-2%	-2%	-3%	-4%	-4%	-4%	-4%	-4%	-6%	-6%	-5%	-5%	-6%
250	0%	0%	0%	0%	-1%	-1%	-2%	-2%	-3%	-4%	-4%	-4%	-5%	-5%	-5%	-6%	-6%	-6%	-7%	-7%
500	0%	0%	1%	-1%	-1%	-2%	-2%	-3%	-2%	-3%	-4%	-4%	-4%	-5%	-4%	-5%	-6%	-6%	-6%	-7%
1000	0%	-1%	-1%	-2%	-4%	-3%	-4%	-5%	-4%	-4%	-6%	-6%	-6%	-8%	-6%	-7%	-8%	-7%	-10%	-7%

Table 1: Regeneration efficacy after 20 cycles at varying load densities.

APPENDIX 1:

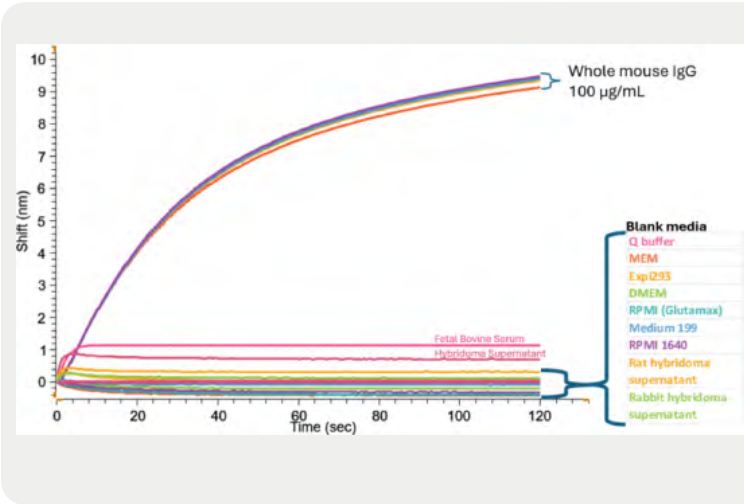
Confirmation of regeneration efficacy

Regeneration efficacy is typically measured by examining the total loading response after 30 seconds loading from the same antibody sample across cycles. In the figure below, we examined the re-usability of the biosensors after 20 cycles of regeneration using Regen Buffer (No Salt) for regeneration buffer and Q Buffer for neutralization buffer. Response is generated using the same antibody sample for each cycle for 30 seconds.

As an additional confirmation of regeneration efficacy, the regenerated surface could also be re-tested with the antigen to determine whether antibody remains bound to the surface. Absence of binding (<0.01 nm in 30 seconds) confirms successful removal of captured antibody and readiness for reuse.

A - Mouse IgG signal vs. Nonspecific binding of media samples

Non-matched prewet



APPENDIX 2:

Preventing non-specific binding (NSB) to mouse hybridoma supernatants

A common concern when working with hybridoma supernatants and other complex media is non-specific binding. The extent of NSB from each of these media onto the MFC XT biosensor surface was evaluated as shown in Figure 4A. Pre-wetting biosensors in a sample with the same composition ("matched pre-wet") substantially reduced these non-specific binding responses, as shown in figure 4B. Diluting samples 1:1 in Q buffer provides an additional reduction in NSB and is recommended as a standard practice regardless of media composition.

B - Mouse IgG signal vs. Nonspecific binding of media samples

Matched prewet

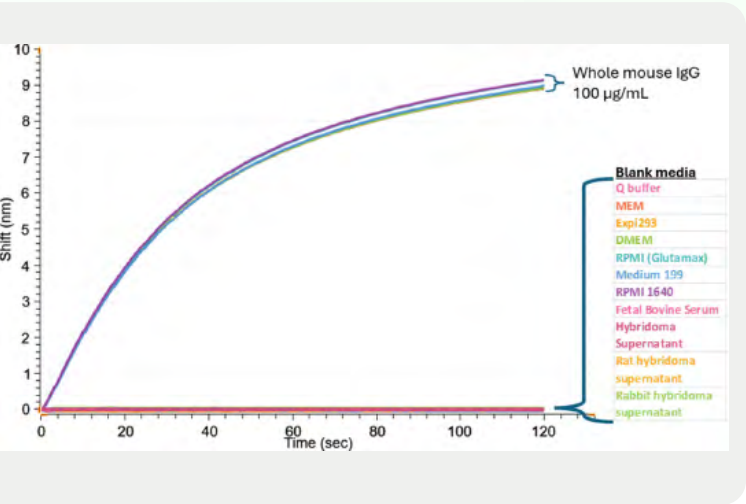


Figure 4: Reduction of non-specific binding from a variety of complex media by pre-wetting in matching media for ten minutes before the assay.

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