PALL ForteBio

# Enabling Depth Filter Selection with Rapid Detection of Monoclonal Antibodies in Culture Using the BLItz System

## OVERVIEW

Harvesting and recovering monoclonal antibodies (mAb) from mammalian cell culture bioreactor samples is just the first step of a closely coordinated chain of purification and processing steps. The ability to provide quick checks at-line for titer provides valuable insight into process variability and opportunities for increased control over them. This technical note demonstrates the utility of the BLItz™ assay system in evaluating mAb recovery post-filtration.

Depth filtration is a precursor to affinity purification, removing cells, cellular debris, and other potential obstructions that could plug a chromatography column. With separation of particles based on fluid travel through a porous media, depth filters are subject to fluid and particle characteristics that will vary from production antibody to antibody and potentially even from batch to batch of the same molecule. The optimal filter for one purpose may not perform as well for another. While pursuing maximum throughput, assessing the mAb recovery pre- and post-filtration ensures that no valuable product is being retained in the filter.

Historically, options to determine titer of a specific mAb in the presence of host proteins present at this stage have been limited. Absorbance measurements at 280 nm only provide the total protein concentration. The time required to identify specific proteins with immunoassay-based techniques like ELISA or HPLC add many hours to the process and delay results.

## PRINCIPLE

The BLItz system from Pall ForteBio operates using Bio-Layer Interferometry to detect real-time binding interaction between molecules. This technique enables the detection of specific mAbs, even in unpurified samples, in as little as 30 seconds. As a single-sample instrument requiring only microvolume amounts of sample, the BLItz system combines affordability and ease-of-use, making it an ideal tool for at-line process development.

Supracap™ depth filter capsules from Pall were used in this evaluation. Supracap capsules are a scalable platform, accommodating from a few liters to a full process. Comprised of Seitz® filter media that meet the highest pharmaceutical quality standards, Supracap capsules offer high flow rates with minimal hold-up volumes. Flexible to fit multiple configurations, they can be assembled in-line or T-style, with a variety of inlet and outlet connections, including sanitary flange and hose barb.

## MATERIALS REQUIRED

- BLItz System (Pall ForteBio, part no. 45-5000)
- Protein A Biosensors (Pall ForteBio, part no. 18-5010)
- Sample Diluent (Pall ForteBio, part no. 18-5028)
- Supracap 100 Depth Filter Capsule (Pall, part no. NP7PDK59)

## METHODS

### Collection of Filter Flow Through

In assessing the fitness-for-use of an existing depth filter product with a new molecule, a key question is whether the filter can be used with minimal loss of product. For this study, the Supracap 100 Depth Filter Capsule was used to clarify a human mAb from media.

- Four volumes were filtered to evaluate mAb retention. Aliquots were taken after 0.5, 1.5, 2.0, and 4.5 kg of material were passed through the Supracap capsule.

## Development of a Standard Curve

To evaluate recovery, relative binding rates obtained on the BLItz system could be compared. For more accurate quantitation, a standard curve needed to be developed. Two approaches were evaluated as proxies. The first used a purified human IgG; the second a partially purified human mAb similar to that in culture.

Standards were diluted 1:3 in Sample Diluent to span a concentration range from 1–700 µg/mL for the purified human IgG, and 0.78–572 µg/mL of the partially purified mAb.

The Create Standard Curve module of the BLItz Pro software was used during data acquisition. The procedure to measure each standard was:

1. A Protein A Biosensor was hydrated in Sample Diluent for at least 10 minutes.
2. 4 µL of sample was added to the drop holder on the BLItz system.
3. The hydrated Protein A Biosensor was loaded onto the BLItz system.
4. Data acquisition was started by closing the cover of the BLItz system. The run time was extended to 60 seconds as a precaution, since the samples had not previously been characterized on the system (Figures 1 and 3).
5. Once the measurement of a sample was complete, the drop holder was rinsed three times with Sample Diluent, and the biosensor discarded before beginning the same sequence of steps for the next sample.
6. After all samples were run, the standard curves (Figures 2 and 4) were saved to a file.

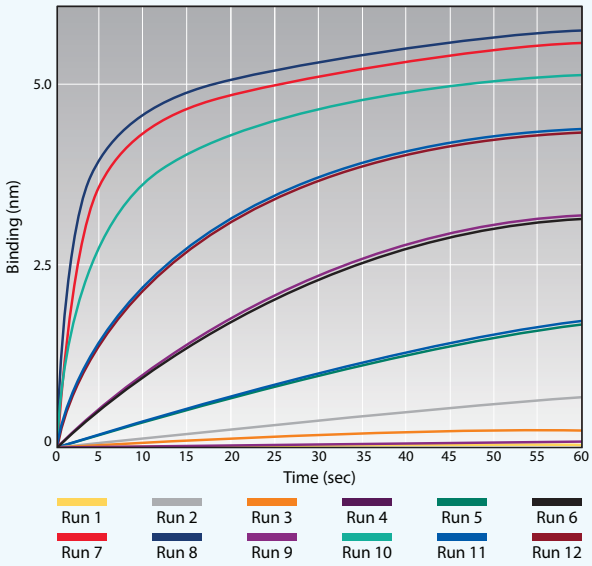


FIGURE 1: Raw run data showing the binding of purified human IgG to Protein A biosensors over time.

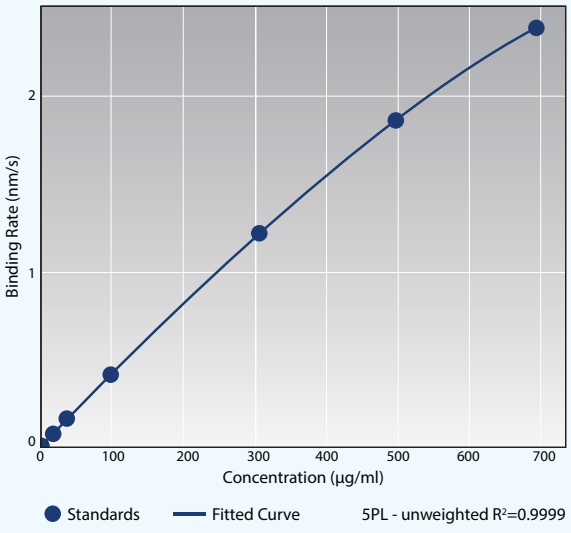


FIGURE 2: Standard curve generated using purified human IgG binding rates.

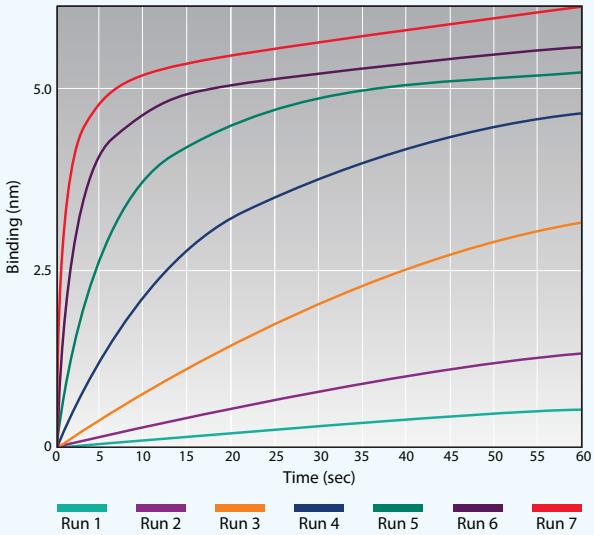


FIGURE 3: Raw run data showing the binding of partially purified human mAb to Protein A biosensors over time.

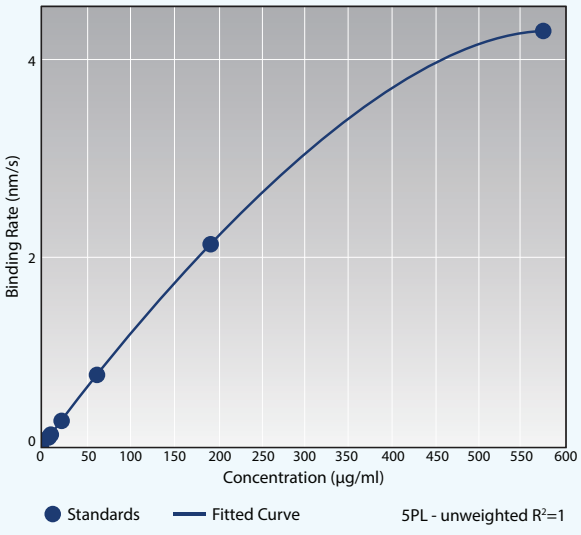


FIGURE 4: Standard curve generated using partially purified mAb binding rates.

### Quantitation from Crude Media

Samples collected pre- and post-filtration were diluted 10x and then analyzed in the same manner, using the Quantitate Sample module of the BLItz Pro software. Concentrations were determined automatically in the software against the previously saved standard curve. Recoveries were calculated based on the calculated titers pre and post-filtration (Tables 1 and 2).

## CONCLUSION

Use of either a standard curve based on a purified IgG or one based on a partially purified mAb yielded similar results. Filter loss in either case was the same — an initial drop at 0.5 kg, which would be expected with a fresh filter and a dilution effect from first rinsing with water. Product loss is minimal, with recovery increasing with increased volumes passed through. The BLItz system detected the mAb in the midst of numerous host cell proteins, enabling a quick at-line assessment of the applicability of a select depth filter for a new molecule.

Selection of a depth filter is just one application where the BLItz system expedites process development by providing quick and specific detection. With a significant speed advantage over other approaches to quantifying mAbs from complex mixtures, the BLItz system's rapid analysis offers potential for better development, monitoring, and control of bioprocessing conditions.

| Volume Through | Concentration (mg/mL) | % Recovery |
|---|---|---|
| Pre-filtration Concentration | 10 | |
| 0.5 kg Post-depth Filtration | 5.3 | 53% |
| 1.5 kg Post-depth Filtration | 9.6 | 96% |
| 2.0 kg Post-depth Filtration | 9.2 | 92% |
| 4.5 kg Post-depth Filtration | 9.8 | 98% |

TABLE 1: Calculated concentrations and recoveries using the purified human IgG standard curve.

| Volume Through | Concentration (mg/mL) | % Recovery |
|---|---|---|
| 0.5 kg Post-depth Filtration | 1.9 | 58% |
| 1.5 kg Post-depth Filtration | 3.1 | 94% |
| 2.0 kg Post-depth Filtration | 3.0 | 91% |
| 4.5 kg Post-depth Filtration | 3.2 | 97% |

TABLE 2: Calculated concentrations and recoveries using the partially purified mAb standard curve. For the lower standard curve range using the partially purified mAb, samples were diluted an additional 3x for accurate interpolation.

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