Rapid Determination of Dynamic Binding Capacity of Resins using Biolayer Interferometry (Octet)

Tri Do, Tim Hotaling, Frank Ho, Laura Lerner, and Lucille Chang – Raven biotechnologies, South San Francisco CA 94080 USA

Raven™ biotechnologies, inc.

Introduction

Determining the dynamic binding capacity of resins is an essential step in optimization of a purification process. A major bottleneck in this optimization is determining where the product breaks through during the loading step.

For the first capture step, the high background absorbance of the load (clarified cell culture supernatant) makes it impossible to use A280 to detect product breakthrough. Unless the product is tagged, it is necessary to use a product-specific ELISA to determine where the product appears in the flow-through. Here we demonstrate the use of a new technology, developed by ForteBio, Inc., that can be used to rapidly quantitate immunoglobulins, even in crude supernatants. We have used this approach to compare the dynamic binding capacity of three protein A resins for a recombinant chimeric antibody.

Materials and Methods

Cell culture supernatants were generated from transfected Chinese hamster ovary (CHO) cells in 2L bioreactors, clarified after harvest by depth filtration, and concentrated by tangential flow filtration. The clarified, concentrated supernatants were sterile-filtered (0.2 µm) prior to loading the column.

Resins being tested were packed to a bed height of 12 mm in 6.6 Omnifit columns. Columns were pre-equilibrated with PBS, pH 7.2 (10 CV). Clarified, concentrated supernatants were loaded at 1.7 mL/min. The flow rate was held at 1.7 mL/min throughout the run. Flowthroughs were collected in 2 mL fractions. Columns were washed with PBS, pH 7.2 (10 CV), and eluted with 0.1 M glycine, pH 2.8. Eluates were collected based on A280 readings. Columns were regenerated with 3 M KSCN and stored in 20% EtOH.

To measure IgG content, aliquots of 200 µL were placed in the wells of 96-well microtiter plates. Standards (polyclonal human IgG) were purchased from ForteBio, and spanned a range from 5 µg/mL to 200 µg/mL. Samples were measured in triplicate.

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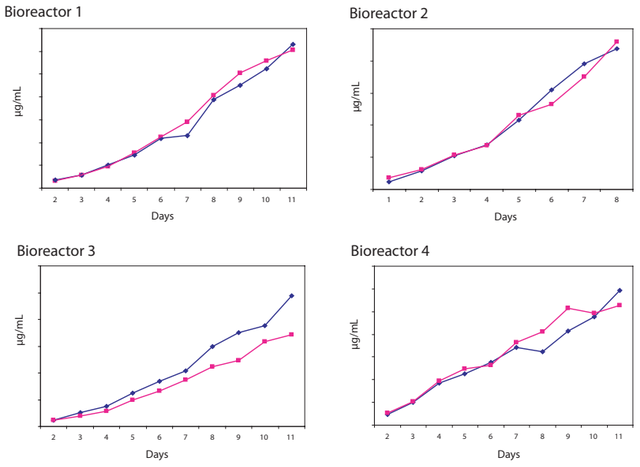
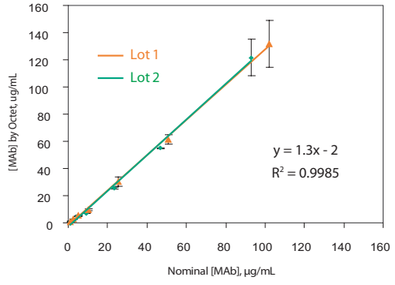
Figure 1.

During qualification of the Octet assay for human IgG, we performed several comparisons between ELISA and Octet values determined on the same samples. Representative results are shown in Figure 1, for CHO supernatants from a set of four bioreactors. In general, we observed excellent agreement between IgG titers determined by ELISA and by Octet.



Comparison of IgG titers for four bioreactors determined by ELISA (pink) or Octet (blue)

Figure 2.



Effect of Polyclonal vs. Monoclonal IgG Standards:
Nominal vs. Measured [IgG], µg/mL, for two lots of Raven MAb.
Nominal = based on Raven reference standard;
Measured = based on ForteBio standards

Figure 3.

Chromatogram of AKTA run using rProtein A Sepharose FF

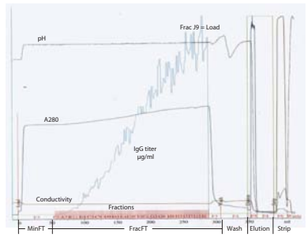


Figure 4.

Chromatogram of AKTA run using MAb Select Xtra

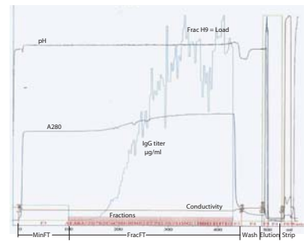


Figure 5.

Chromatogram of AKTA run using MAb Select Sure

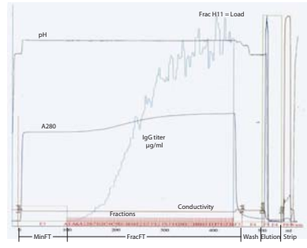
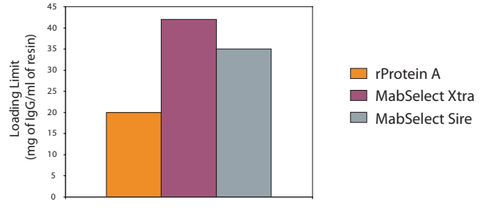


Table. Mass balance for column chromatography

	Recovery (%)		
Samples	**rProtein A Seph FF**	**MabSelect Xtra**	**MabSelect Sure**
Load			
MinFT	3	3	3
FracFT	35	22	46
Eluate	65	71	57
Accountability	103	96	106

Figure 6.



Comparison of dynamic binding capacities of three protein A resins for Raven MAb, determined by Octet

Discussion

We compared the polychonal ForteBio standards with an internal Raven monoclonal IgG standard. Raven IgG samples gave, on average, 30% higher measured concentrations when based on ForteBio standards. The difference in response probably reflects the difference in binding rates between a pool of IgG types (ForteBio standards) and a monoclonal isotype (Raven standard). As with any quantitative assay, it is important to choose the appropriate reference standard to get the most accurate results.

Conclusions

- A plate of 96 samples can be analyzed in less than 20 minutes with an Octet instrument. In contrast, other methods for determining product breakthrough, such as ELISA, SDS-PAGE, or dot-blots, are more time-consuming and labor-intensive.
- There is good agreement between the ELISA and Octet methods. In our hands, the typical %sd for triplicate determinations by Octet is 5-15%, depending on concentration.
- Bio-layer interferometry technology allowed us to determine IgG concentration for about 200 samples in about an hour. In contrast, determining the concentration by ELISA would have taken one or two days. The ELISA is much more sensitive than the Octet method, so it requires time-consuming serial dilutions.