Quantitation of Residual Protein A and MabSelect SuRe using a "Dip and Read" Assay

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Abstract

Detection of residual Protein A in product purified using a Protein A, or similar column matrix such as MabSelect SuRe™, is a critical quality control step in antibody therapeutic development and production. During the IgG purification process, Protein A can leach from the purification column and co-elute with the IgG. This contaminant in the therapeutic mixture can cause adverse reactions in patients and thus must be detected and minimized. The Octet family of instruments utilizes a unique label-free "dip and read" detection technology to quantify biomolecules in solution. ForteBio has developed a new kit for use on this system to detect and quantify residual Protein A. The new Dip and Read™ Residual Protein A Detection Kit contains biosensors and all the reagents required to quantitate Protein A and MabSelect SuRe. The data presented here demonstrates that the Octet system can accurately and precisely detect 100 pg/mL Protein A or MabSelect SuRe in bioprocess samples.

Materials and Methods

The experiments described here were performed using the following materials and instruments:

- Dip and Read™ Residual Protein A Detection Kit (P/N 18-5075)
- Sidekick™ Biosensor Immobilization Station (P/N 30-5011)
- Octet™ RED System (P/N 30-5036)

The Octet platform of instruments uses Biolayer Interferometry technology to detect and quantify biomolecular interactions (Figure 1). The method used to prepare and analyze the samples described here is described Figure 2 and in detail in Technical Note number 18 "Dip and Read™ Residual Protein A Detection Kit" available at www.fortebio.com/literature.

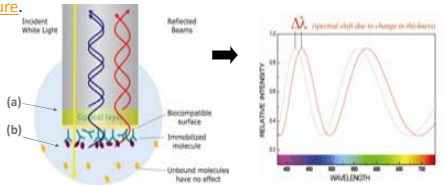


Figure 1: Principals of Biolayer Interferometry (BLI)
Octet provides real-time monitoring of biomolecular interactions using BioLayer Interferometry (BLI) technology. Any change in the number of molecules bound to the biosensor tip changes the optical layer thickness which causes a shift in the interference pattern. Adding molecules (binding) to the molecular layer increases the thickness of the biological layer and results in the interference pattern shifting to the right. Reducing the thickness of the layer (dissociation) shifts the wavelength peaks to the left.

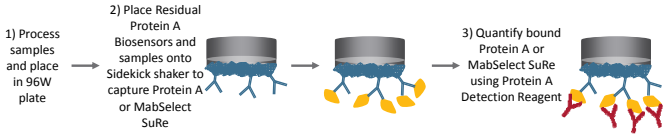
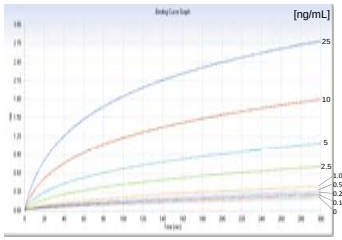


Figure 2: Typical workflow for the Dip and Read Residual Protein A Assay
1) Process samples using supplied Denaturation Buffer and heat treatment
2) Load samples onto Residual Protein A Biosensors using Sidekick shaker for 1 hour
3) Place loaded biosensors into Octet RED to quantify bound Protein A or MabSelect SuRe using the supplied Protein A Detection Reagent. Instrument read time = 5 minutes/column of 8 samples on Octet RED or 5 minutes per 16 samples on the Octet RED384 and QK384.
4) Analyze data using Octet Data Analysis software

Fast. Accurate. EASY.

Results: Accuracy



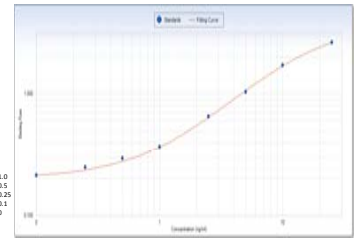


Table 1: Recombinant Protein A Spiked Concentration (ng/mL); All Dilutions include 0.5 mg/mL human IgG	Calculated Concentration (ng/mL)	% Recovery N = 3
0.10	0.11	110
0.25	0.23	92
0.50	0.44	88
1.0	0.90	90
2.5	2.31	92
5.0	4.86	97
10.0	11.1	111

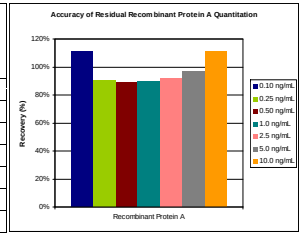


Figure 3 and Table 1: Recovery of Recombinant Protein A
Recombinant Protein A from Pierce Chemical Company (P/N 21186) was spiked into ForteBio Sample Diluent at various concentrations and processed independently in triplicate in the presence of 0.5 mg/mL human IgG. Figures show binding curves for Recombinant Protein A standards and the calculated standard curve. Standards were fit using an unweighted 4PL standard curve equation.

Table 2: MabSelect SuRe Spiked Concentration (ng/mL)	Calculated Concentration (ng/mL)	% Recovery N = 3
0.10	0.11	111
0.25	0.23	92
0.50	0.49	98
1.0	0.98	98
2.5	2.37	95
5.0	5.36	107
10.0	11.8	118

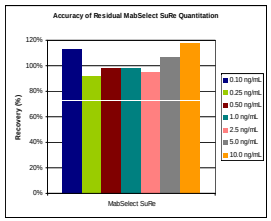


Table 2: Recovery of MabSelect SuRe
MabSelect SuRe from GE Healthcare (P/N 28-4018-60) was spiked into ForteBio Sample Diluent at various concentrations and processed independently in triplicate.

Results: Sensitivity

Standard deviation of the binding rate of the buffer only control in the presence of 0.5 mg/mL human IgG (N= 16)	LOD (3*SD of Buffer Only Control) Determined in the presence of 0.5 mg/mL human IgG
0.0144	43.2 pg/mL

Table 3: Limit of Detection (LOD)
Buffer only controls (N = 16) were processed and analyzed on the Octet RED. The LOD was calculated using the equation LOD = 3*SD of the resulting data.

Results: Precision

Table 4: Recombinant Protein A Spiked Concentration (ng/mL); All Dilutions include 0.5 mg/mL human IgG	Calculated Concentration CV (%) N = 3
0.10	8.9
0.25	12.9
0.50	10.3
1.0	9.0
2.5	5.4
5.0	5.1
10.0	7.9

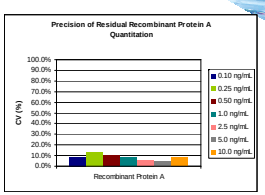


Table 4: Precision of residual Recombinant Protein A quantitation
Recombinant Protein A from Pierce Chemical Company (P/N 21186) was spiked into ForteBio Sample Diluent at various concentrations and processed independently in triplicate in the presence of 0.5 mg/mL human IgG.

Table 5: MabSelect SuRe Spiked Concentration (ng/mL)	Calculated Concentration CV (%) N = 3
0.10	10.1
0.25	8.4
0.50	6.5
1.0	5.0
2.5	6.5
5.0	6.8
10.0	12.1

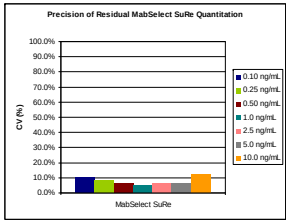


Table 5: Precision of residual MabSelect SuRe quantitation
MabSelect SuRe from GE Healthcare (P/N 28-4018-60) was spiked into ForteBio Sample Diluent at various concentrations and processed independently in triplicate.

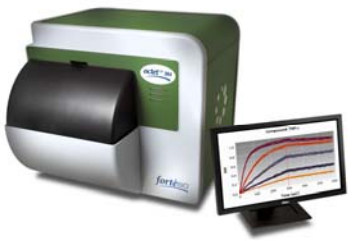
Summary:

The Dip and Read Residual Protein A Detection Kit provides:

- **A very accurate quantitation (% Recovery ~ 90-110%) of both Recombinant Protein A and MabSelect SuRe**
- **A highly sensitive assay (LOD below 100 pg/mL) for both residual Recombinant Protein A and MabSelect SuRe in the presence of 0.5 mg/mL human IgG**

The Octet platform provides

- **Precise measurement of both Recombinant Protein A and MabSelect SuRe**
- **Unmatched ease of use in biotherapeutic development and bioprocess monitoring**



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