In the biopharmaceutical industry, downstream processing for monoclonal antibody production typically includes three chromatographic steps: capture, intermediate purification, and polishing. Protein A is frequently used as a capturing step, which results in excellent throughput (i.e., capacity and speed) while playing an important role in concentrating the target molecule—immunoglobulin. To monitor monoclonal antibody titer and yield from cell culture supernatants before expensive preparative and large amounts of protein A are employed, a small (analytical) scale procedure is necessary to determine the titer of monoclonal antibody for the optimal time for harvest of the monoclonal antibody product. In this note, pre-packed Agilent Bio-Monolith Protein A columns were used to illustrate the quick capture of monoclonal antibody titer from cell supernatant.

Experimental

Conditions
Column: Agilent Bio-Monolith Protein A (μ/l 50569.3639)
Sample: 100 µL of 20/mL mg E. coli protein (cell lysate) was mixed with 100 µL humanized IgG1 (2.5 mg/mL). Escherichia coli lysate and E. coli lysate mixed with IgG1 were mixed with eluent A and injected separately onto a Bio-Monolith Protein A column.
Eluent: A: 0.5 mM sodium phosphate, pH 7.4; B: 0.1 M citric acid, pH 2.8
Injection: see chromatograms
Flow rate: 1.0 mL/min
Gradient:
Time (min) Ejection B (%) 0 0 0.5 0 6.6 100 1.7 100 1.8 100 2.5 0 0
Detector: UV, 280 nm
System: 1200 Infinity Series

Results and Discussion

Quantifying titer of monoclonal antibody
To demonstrate the ability of the Bio-Monolith Protein A column in the quantification of IgG1, different concentrations of IgG1 mixed with E. coli supernatant were injected onto the column. The chromatogram in Figure 3 shows various concentrations of IgG1. The area of these injections were also plotted versus their concentrations, as seen in Figure 2 insert. The data indicated a linear relationship between area and concentration. The limit of detection for these columns has not been established.

Figure 2. Agilent Bio-Monolith Protein A column quantitates monoclonal antibody from a harvested cell culture mixed with IgG1.

Effect of flow rate on IgG1 binding
Different flow rates were used with the column to understand the effect of flow rate on the IgG1 binding and column performance. There was a very little effect of IgG1 binding on the column when the flow rate was increased (Table 1). The percentages of unbound proteins (E. coli proteins) relative area and bound IgG1 relative area remain unchanged at different flow rates. The decrease of the area of IgG1 and unbound proteins (mAU/s) at high flow rates is due to the use of the same data collection rate of the detector.

Table 1. Flow rate versus peak relative area on unbound proteins and IgG1

<table>
<thead>
<tr>
<th>Flow rate (mL/min)</th>
<th>Unbound area (mAU/s)</th>
<th>IgG1 area (mAU/s)</th>
<th>Unbound relative Area (%)</th>
<th>IgG1 relative area (%)</th>
<th>Pressure [bar]</th>
</tr>
</thead>
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<tr>
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</table>

Salt tolerance
Cell culture supernatant often contains neutral salts such as sodium chloride (NaCl) and potassium chloride (KCl) for protein stabilization. Salt concentrations ranging from 100 to 200 mM are typically used. However, salt is a strong eluting solvent for many purification processes, including affinity and ion-exchange methods. For some methods, little as 50 mM NaCl can elute bound proteins from the column and prevent the column from retaining proteins for separation. Therefore, it is important to demonstrate that the affinity Bio-Monolith Protein A column can tolerate a certain amount of salt. Figure 4 indicates that the column could tolerate samples with high salt concentration without deterioration of peak shapes. Furthermore, calculation of peak areas from all three concentrations indicated that any change was negligible.

Figure 3. The binding of IgG1 with the column was evaluated at several flow rates

Figure 4. IgG1 and E. coli supernatant sample mixed with different salt concentrations and injected onto the Agilent Bio-Monolith Protein A column to evaluate the column’s ability to work with samples containing salt.

Conclusions
The Agilent Bio-Monolith Protein A column has very high affinity for monoclonal antibody. It is evident that it can capture, separate, and elute monoclonal antibody from supernatant in less than 1.4 minutes, and quantitate monoclonal antibody to assess harvesting time. The column tolerates high concentrations of salt without affecting its performance, operates well at different buffer pH, and has high reproducibility.