

# Protein A Affinity Column for Monoclonal Antibody (MAb) Titer Analysis

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## Overview

**Purpose:** Demonstrate the capability of a Thermo Scientific™ MAbPac™ Protein A column for titer analysis of monoclonal antibodies (MAb).

**Methods:** The MAbPac Protein A column has been analyzed on a Biocompatible HPLC system. Chromatographic conditions such as flow rate, column temperature, and sample loadings were tested to evaluate the column performance.

**Results:** The MAbPac Protein A column can accurately determine the MAb titer in the range of 0.025 mg/mL to 5 mg/mL. The total analysis time is less than 2 minutes.

## Introduction

Early in the development of recombinant monoclonal antibodies (MAbs), a large number of harvest cell culture (HCC) samples must be screened for IgG titer. Affinity chromatography employing a Protein A ligand is often used to determine the MAb concentration as well as to purify it for downstream aggregate and charge variant analysis. The challenge facing the analytical laboratories in the pharmaceutical industry is to develop high-throughput and robust titer assay.

In the current study, we are presenting a Protein A column for fast MAb titer analysis. The column is developed based on a novel polymeric resin. The hydrophilic surface is designed to accommodate protein conjugation. A recombinant Protein A ligand is covalently attached onto the hydrophilic resin surface. The functionalized resin with recombinant Protein A is then packed into a 4 × 35 mm PEEK™ column body.

The hydrophilic nature of the backbone minimizes non-specific binding and therefore enables accurate quantification of the MAb titer. In addition, the non porous particle produces a highly efficient column at high flow rates. When injecting 20 µg of rabbit IgG, the IgG peak width at half height is about 0.01 min. The sharp peak shape also provides great sensitivity. As little as 0.25 µg of MAb can be easily detected. The Protein A column has very low back pressure and this allows high flow rate for fast analysis. At 2.5 mL/min, the entire analysis, including equilibration, takes only 1.6 min. Ruggedness testing shows that this column can go through more than 2,000 cycles with very little loss of performance. The HPLC compatibility of this column allows automation, provides accurate and high throughput analysis.

## Methods

### Sample Preparation

Monoclonal antibody harvest cell culture was a gift from a local biotech company. The HCC was filtered through a 0.22 µm membrane prior to sample injection.

### Columns

MABPac Protein A column, 12 µm, 4 × 35 mm

### Buffers

Eluent A: 50mM Sodium Phosphate, 150 mM NaCl, 5% acetonitrile, pH 7.5

Eluent B: 50mM Sodium Phosphate, 150 mM NaCl, 5% acetonitrile, pH 2.5

### Liquid Chromatography

HPLC experiments were carried out using an hybrid system equipped with:

Thermo Scientific™ Dionex™ ICS-3000 Dual Gradient Pump System

Thermo Scientific™ Dionex™ TCC-100 Column Compartment

Thermo Scientific™ WPS-3000 Pull-Loop AutoSampler

Thermo Scientific™ Dionex™ VWD-3400RS UV Detector equipped with a 2.5 µL Micro Flow Cell

20 µg rabbit IgG was injected every 100 cycles over the course of 2,000 runs, see Figure 6. Retention time, peak area, and peak width of IgG from each chromatographic run are listed on the inserted table.

**TABLE 1. Effect of Flow Rate on IgG peak area.**

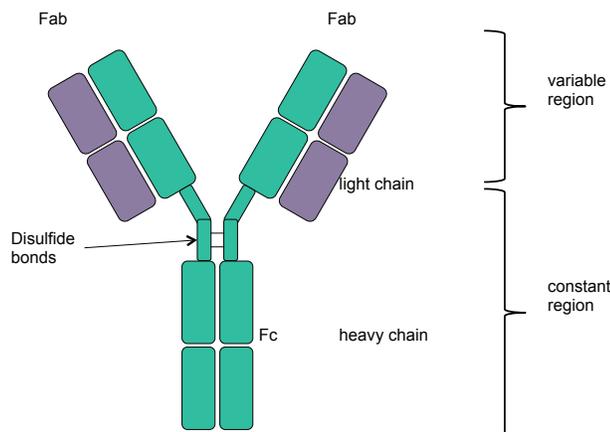
Flow Rate (mL/min)	Total Area (mAu.min)	Unbound Area (mAu.min)	Unbound Relative Area, %	IgG Area (mAU.min)	IgG Relative Area, %
2.5	7.381	1.196	16.20	6.185	83.80
2.0	8.826	1.419	16.08	7.407	83.92
1.5	12.111	2.068	17.08	10.043	82.92
1.0	17.895	2.937	16.41	14.958	83.59
0.5	34.219	5.582	16.31	28.637	83.69

## Results

Staphylococcal protein A (SPA) plays an important role in immunology and biochemistry owing to its specific interaction with the Fc part of immunoglobulin G (IgG) from many mammals. SPA is a cell wall associated protein domain exposed on the surface of the Gram-positive bacterium, *Staphylococcus aureus*. SPA consists of three different regions; S, being the signal sequence that is processed during secretion, five homologous IgG binding domains E, D, A, B, and C, and a cell-wall anchoring region XM. SPA and the smaller ligands derived from SPA have been used widely for the affinity purification of antibodies.<sup>1</sup>

Recombinant Protein A (rSPA), is expressed in *E. coli* as opposed to the native protein extracted from *Staphylococcus aureus*. rSPA is a 45 kDa protein containing the same amino acid sequence and molecular mass as the native Protein A sourced from *S. aureus*. rSPA is used as the affinity ligand for the MAbPac Protein A column.

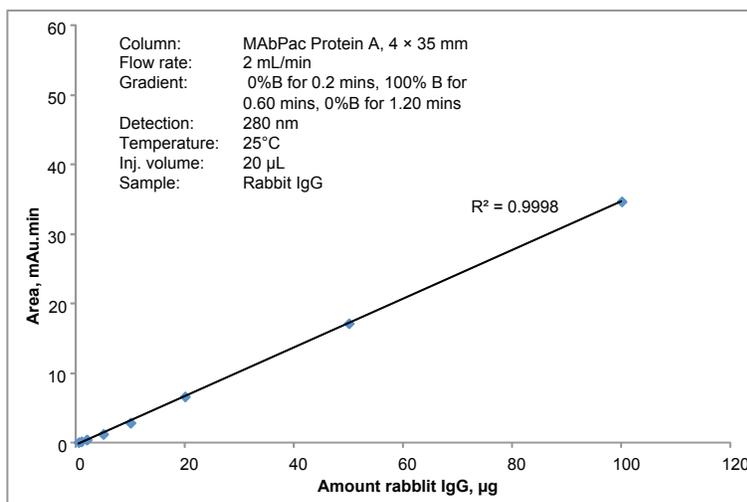
**FIGURE 1. Schematic of an antibody showing the fragment crystallizable region (Fc) and the fragment antigen binding region.**



### Dynamic loading capacity

The dynamic loading capacity of rabbit IgG, isolated from pooled normal serum, is no less than 100  $\mu\text{g}$  on the MAbPac Protein A column, analyzed at a flow rate of 2 mL/min. Figure 2 shows the linearity of area to sample load for rabbit IgG when loaded onto the MAbPac Protein A column. This correlation allows the MAbPac Protein A column to be used for quantitation of MAb in harvest cell culture over a wide range of concentrations.

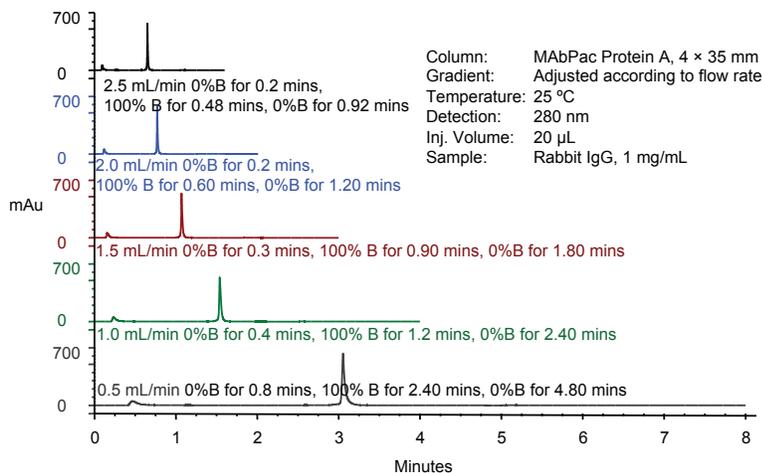
**FIGURE 2. Area dependence on rabbit IgG Loading.**  
 Gradient: 0% B for 0.2 mins, 100% B for 0.6 mins, 0% B for 1.2 mins.



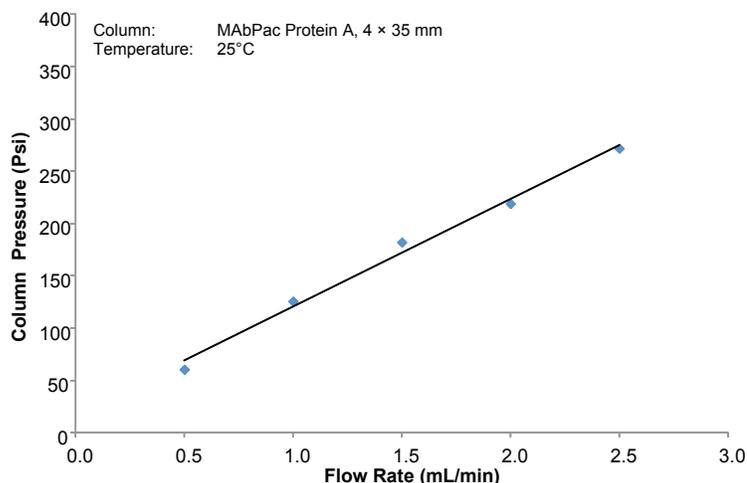
**Influence of flow rate on column pressure, cycle time and peak area**

The MAbPac Protein A column can be used at a flow rate up to 2.5 mL/min. The recommended flow rate is 2 mL/min. Figure 4 shows the effect on increasing flow rate on column backpressure. Figure 3 shows that as you increase the flow rate there is little effect on the amount of IgG that binds to the column. The increase in total area (Table 1) is due to the use of the same data collection rate of the detector.

**FIGURE 3. Effect of flow rate on IgG binding.**



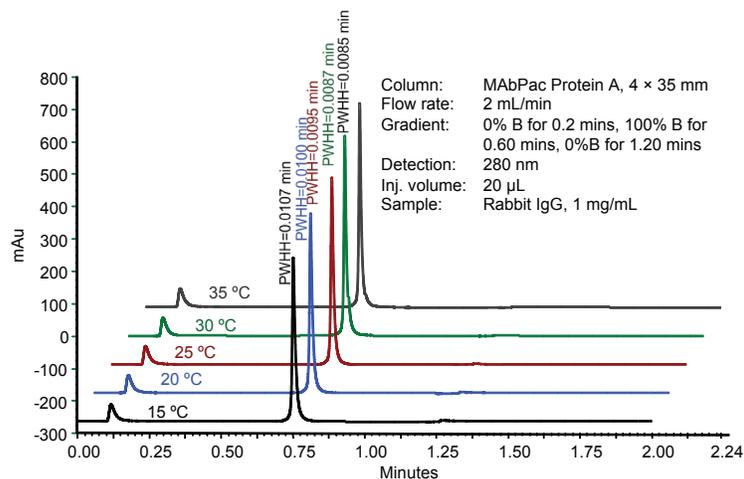
**FIGURE 4. Example of effect of flow rate on column backpressure.**



### Influence of temperature on binding efficiency

It is recommended to use the MAbPac Protein A column at temperatures no higher than 35°C. To ensure data consistency and to prolong the column life time, it is recommended to use a column oven to control the temperature to 25°C. Figure 5 shows how rabbit IgG binding is minimally affected by temperature. Proteins with different binding association may be affected differently.

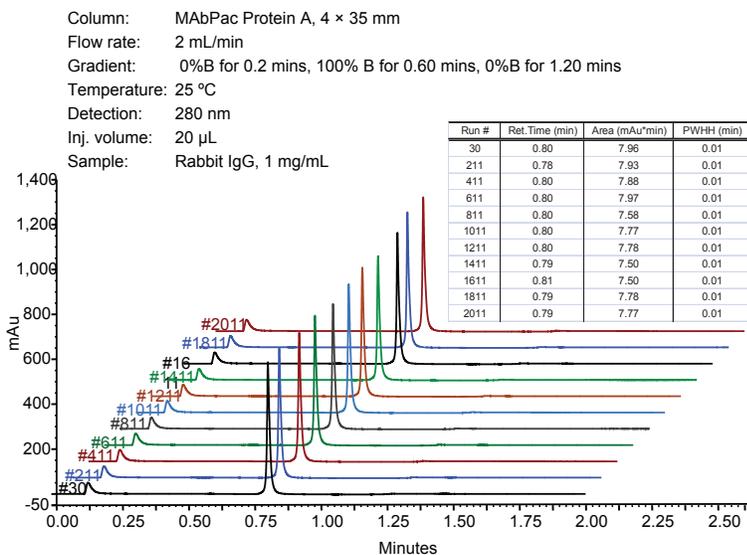
**FIGURE 5. Temperature effect on binding efficiency.**



### Column Ruggedness

The MAbPac Protein A column has been tested continuously for 2,000 cycles. Every hundred cycles, a set of calibration standards (from 0.01 mg/mL to 5 mg/mL) was analyzed. As shown in Figure 6, the retention time, peak area, and peak width of IgG remain unchanged. In the upper range, there is no loss of binding capacity and in the lower range sensitivity is maintained.

**FIGURE 6. Chromatograms of rabbit IgG analyzed on the MAbPac Protein A column.**

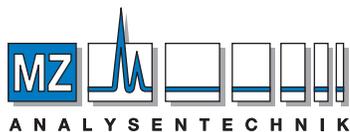


## Conclusions

- The MAbPac Protein A column has a dynamic loading capacity of at least 100 µg. It is capable of quantifying MAbs in the range of 0.01 mg/mL to 5 mg/mL.
- The MAbPac Protein A column has a fast cycle time. At 2 mL/min, a complete titer analysis takes 2 min.
- The MAbPac Protein A column has been successfully tested through 2,000 cycles without loss of binding capacity.

## References

1. Hober, S., Nord, K., and Linholt, M. Protein A Chromatography for Antibody Purification. *Journal of Chromatography B*, 848 (2007) 40–47.



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