

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

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## Key Words

MABPac Protein A, affinity chromatography, MAb purification, monoclonal antibody, MAb, titer analysis, immunoglobulin G (IgG)

## Abstract

The Thermo Scientific™ MABPac™ Protein A column is a high-performance affinity chromatography column specifically designed for the determination of monoclonal antibody concentration in harvest cell culture. The non-porous, hydrophilic stationary phase enables fast and accurate MAb titer analysis. The ability to collect the IgG fraction during the HPLC analysis allows the analyst to characterize the IgG charge variants using a novel pH gradient approach.

## Introduction

Early in the development of recombinant monoclonal antibodies (MAbs) for biotherapeutics, a large number of harvest cell culture (HCC) samples must be screened for immunoglobulin G (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 [1]. With a high number of clones and samples to characterize, the challenge facing the biotherapeutic industry is the development of fast and reproducible automated platform methods for MAb titer and characterization. In the current study, we present the MABPac Protein A column for fast MAb titer analysis, IgG peak fractionation, and subsequent charge variant analysis on a cation exchange column using a pH gradient.

Monoclonal antibody titer analysis is required for accurate determination of monoclonal antibody quantities, including HCC clone production yields. The unique MABPac Protein A column resin has been optimized to provide fast, accurate separation over a wide linear range. Results are quickly obtained, eliminating the need for multiple injections or re-analysis. The HPLC compatibility of this column, in combination with low back pressure and high efficiency, allows automation to provide higher throughput and more accurate analysis. The column format is designed for rapid automation of loading, binding, elution, and collection using Thermo Scientific biocompatible systems.



The MABPac Protein A column is based on a novel, non-porous polymeric resin with a particle size of 12  $\mu\text{m}$  and a hydrophilic surface, which 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  $\times$  35 mm PEEK column body.

The hydrophilic nature of the backbone minimizes non-specific binding and enables accurate quantification of the MAb titer. In addition, the small particle size of the pellicular resin produces a highly efficient column. When injecting 20  $\mu\text{g}$  of rabbit IgG, the IgG peak width at half height is approximately 0.01 min. The sharp peak also provides enhanced sensitivity and in this experiment 0.25  $\mu\text{g}$  of MAb was easily detected.

The MAbPac Protein A column generates very low backpressure, facilitating the use of high flow rates and therefore fast analysis. At 2.0 mL/min, the entire analysis, including equilibration, takes only 2 minutes. Under these conditions, ruggedness testing shows that this column can withstand more than 2,000 cycles with very little loss of performance.

In addition to titer analysis, the MAbPac Protein A column is also capable of purifying sufficient amounts (up to 100 µg) of IgG from harvest cell cultures for laboratory scale characterization. In the example presented, a typical workflow is demonstrated in which the collected fraction is re-injected onto a strong cation exchange column for charge variant analysis using a linear pH gradient.

## Experimental Details

Consumables	Part Number
All chemicals and rabbit IgG were purchased from reputable suppliers. Monoclonal antibody harvest cell culture was a gift from a local biotech company.	
The HCC was filtered through a Thermo Scientific™ Nalgene™ 0.22 µm membrane prior to sample injection.	292-4520
The Thermo Scientific CX-1 pH Gradient Buffer Kit includes 125 mL of 10X buffer A concentrate (pH 5.6) and 125 mL of 10X buffer B concentrate (pH 10.2).	083274

Columns	Part Number
MAbPac Protein A, 4 × 35 mm	082539
MAbPac SCX-10, 10 µm, 4 × 250 mm	074625

### Liquid Chromatography

HPLC experiments were carried out using a Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS System equipped with:

SRD-3600 Membrane Degasser

DGP-3600RS Biocompatible Dual-Gradient Rapid Separation Pump

TCC-3000SD Thermostatted Column Compartment with two biocompatible 10-port valves

WPS-3000TBRS Biocompatible Rapid Separation Thermostatted Autosampler

VWD-3400RS UV Detector equipped with a Micro Flow Cell

PCM-3000 pH and Conductivity Monitor

### Eluents for MAbPac Protein A (unless otherwise stated)

Eluent A: 50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 7.5

Eluent B: 50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 2.5

### Eluents for MAbPac SCX-10

Eluents A and B each were prepared by diluting the corresponding 10X buffer concentrate 10 fold using deionized water.

### Gradient for MAbPac Protein A

0–0.2 min, 0% B

0.2–0.8 min, 100% B

0.8–2 min, 0% B

### Gradient for MAbPac SCX-10

0–1 min, 0% B

1–31 min, 0–100% B

31–34 min, 100% B

34–40 min, 0% B

### Data Processing

Thermo Scientific™ Dionex™ Chromeleon™ software version 6.8

## Results

### Faster MAb Titer Analysis

Fast and accurate results are important in titer analysis. Figure 1 shows a 10  $\mu$ L injection of HCC sample onto the MAbPac Protein A column. The unbound material elutes first (large peak). Next, the MAb is released using a low pH wash (pH 2.5). The proprietary hydrophilic resin, with its fast mass transfer capability, results in sharp, concentrated peaks and therefore fast, efficient elution. The complete cycle time, including equilibration, is 2 minutes. The MAb titer is determined by back calculating the integrated IgG peak area against a previously generated calibration curve (see Figure 1 insert). The unique surface chemistry of the MAbPac Protein A provides accurate titer analysis with linearity over a wide concentration range (0.025 mg/mL to 5 mg/mL).

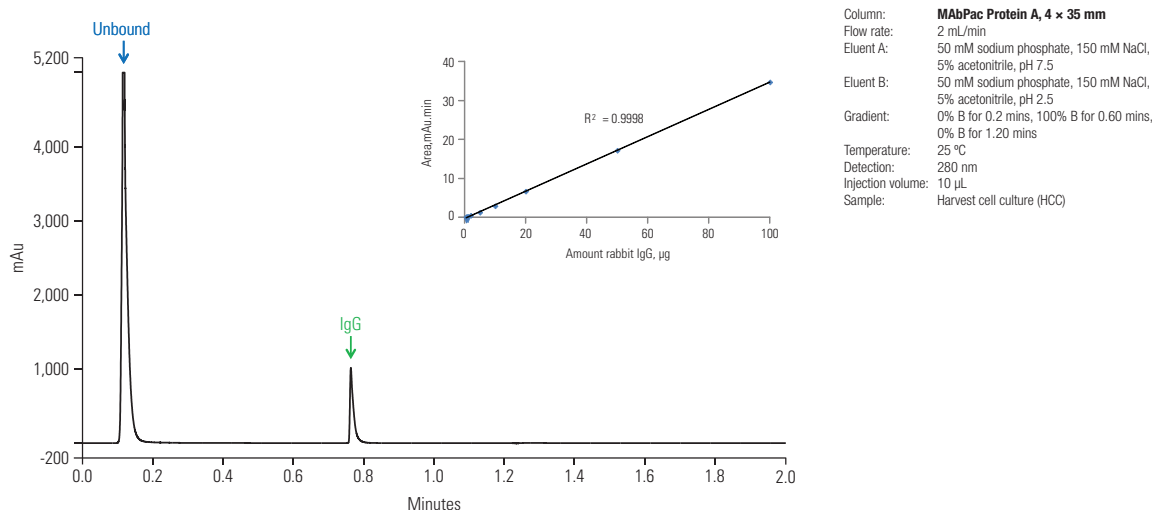


Figure 1: Analysis of HCC on the MAbPac Protein A (4 x 35 mm) column

### Purified MAb

Affinity chromatography is also a useful tool for concentrating and purifying IgG material for later experiments or second dimension (2D) chromatography, such as size exclusion or ion-exchange analysis. The MAbPac Protein A column resin chemistry is designed for accurate binding and efficient elution of the MAb resulting in a sharp, concentrated sample peak. Figure 2 shows the chromatographic separation of a 50  $\mu$ L injection of HCC. The IgG fraction was collected into a 96-well plate using time-based triggers. At 2 mL/min flow rate, the total volume collected was 200  $\mu$ L; the total collection time was 0.1 min. The MAbPac Protein A column efficiently separates, concentrates, and purifies the MAb.

Figure 3 shows the collected Protein-A purified fraction, analyzed in the second dimension on a MAbPac SCX-10, 10  $\mu$ m column using a linear pH gradient from pH 5.6 to pH 10.2. The chromatogram shows the variants in the purified IgG fraction. Both the first and the second dimension analysis can be carried out on the UltiMate 3000 BioRS system, automated under the control of the Chromeleon Chromatography Data System (CDS), enabling multiple HCC samples cycles without user intervention.

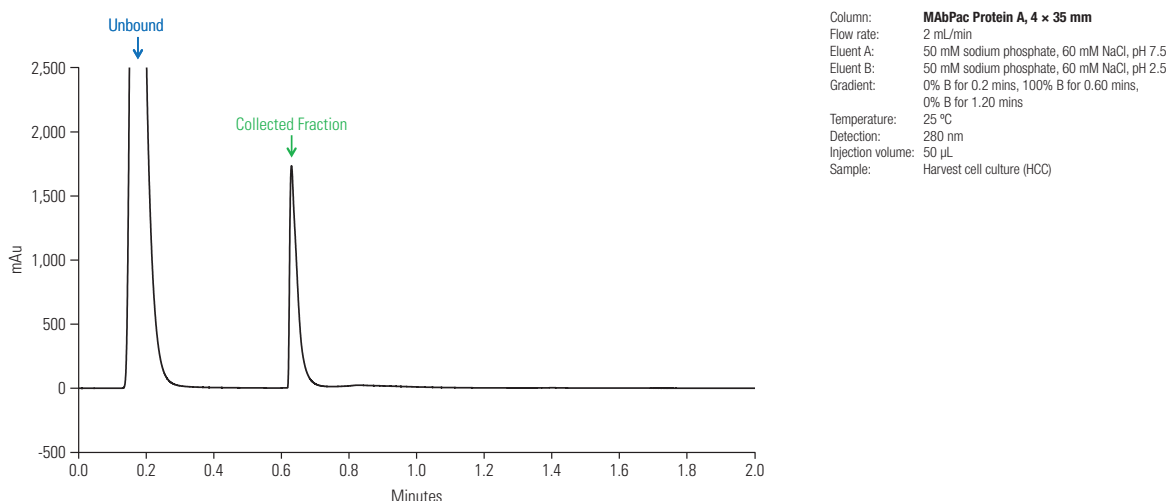


Figure 2: Purification of HCC on the MAbPac Protein A (4 x 35 mm) column

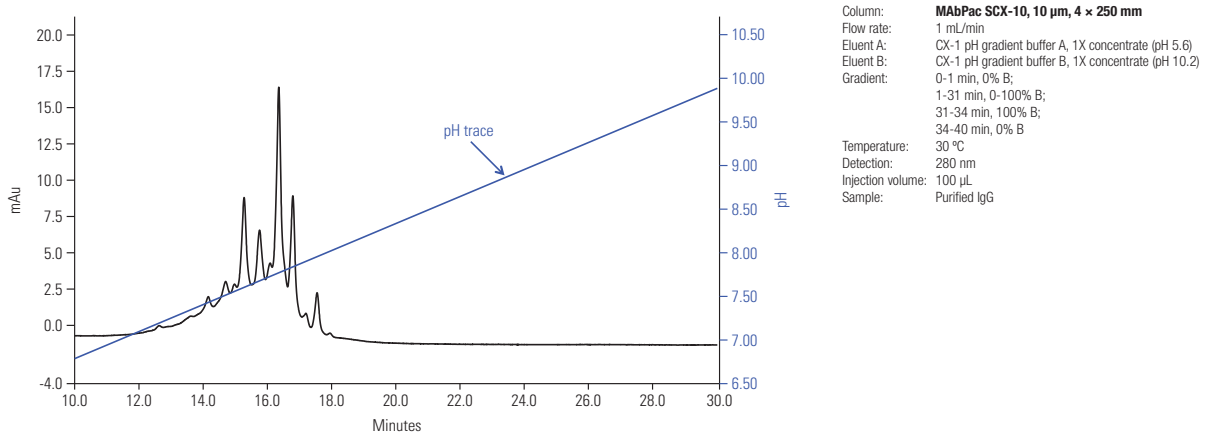


Figure 3: Charge variant analysis on the MAbPac SCX-10 column using linear pH gradient

### Reproducibility

The MAb Pac Protein A column was tested continuously for 2,000 cycles and every hundred cycles a set of calibration standards (from 0.025 mg/mL to 5 mg/mL) was analyzed. As shown in Figure 4, the retention time, peak area, and peak width of IgG remain unchanged. In the upper range at 5 mg/mL, there is no loss of binding capacity, supported by the fact that IgG peak area RSD is less than 1.5%. In the lower range at 0.1 mg/mL, sensitivity is maintained, supported by the fact that IgG peak area RSD is less than 7%.

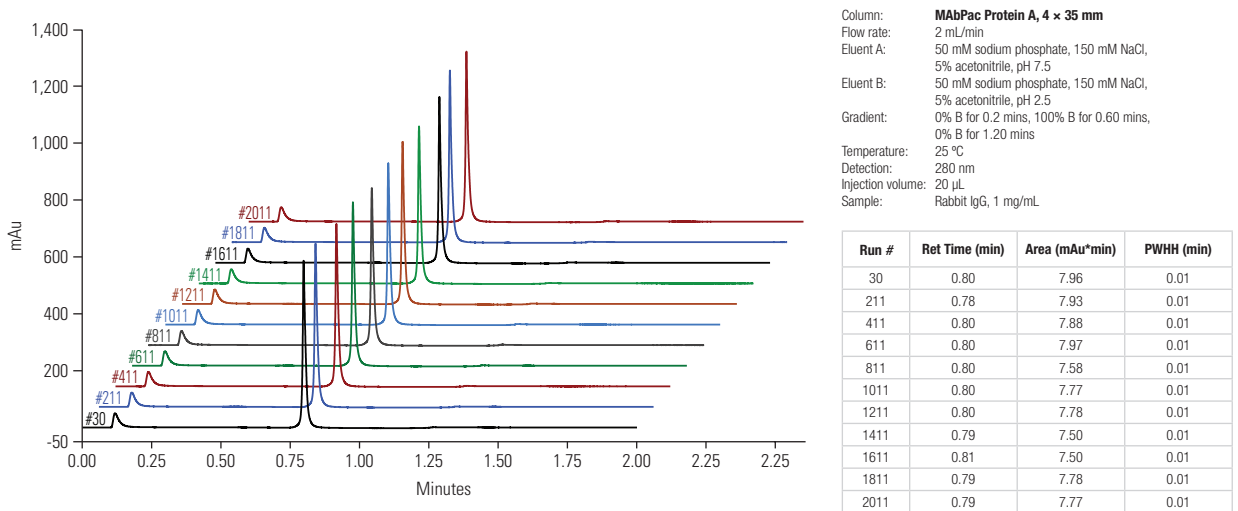


Figure 4: Reproducibility

## Conclusion

- The combination of the UltiMate 3000 Bio RS system and the MAbPac Protein A column can be used in an automated 2D work flow for MAb titer, purification, and subsequent charge variant analysis.
- 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 0.025 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.

## Reference

- [1] Hober, S.; Nord, K.; and Linhult, M. Protein A Chromatography for Antibody Purification. *Journal of Chromatography B*, 2007, 848, 40–47.

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