

# Thermo Scientific MAbPac Protein A Column

High performance affinity column for  
monoclonal antibody titer analysis

The Thermo Scientific™ MAbPac™ Protein A column is a high performance affinity chromatography column specifically designed for fast, accurate determination of monoclonal antibody concentration in the harvest cell culture.

## Product Highlights

- A novel non-porous polymeric resin with a hydrophilic surface
- Functionalized with the recombinant Protein A ligands
- Wide range of MAb quantitation: 0.025 mg/mL to 5 mg/mL
- Fast analysis: 2 min/analysis
- Durability: up to two thousand injections per column
- Low pressure: even at 2 mL/min flow rate



## Introduction

Monoclonal antibodies (MAbs) are a growing family of therapeutic proteins. Early in recombinant monoclonal antibody development, 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. The MAbPac Protein A column is a high pressure liquid chromatography (HPLC) column designed to provide fast, accurate, titer analysis, with linearity over a wide concentration range; without carryover.

## Column Technology

The MAbPac Protein A column is based on a novel non-porous polymeric resin consisting of a hydrophobic divinylbenzene core and a hydrophilic surface. This proprietary resin is designed for fast mass transfer, resulting in efficient separations, without carryover. The recombinant Protein A ligands are covalently attached onto the hydrophilic resin surface through their amine groups, to provide a very rugged, reproducible column. The column is available in a HPLC bio-inert, 4 × 35 mm PEEK column body.

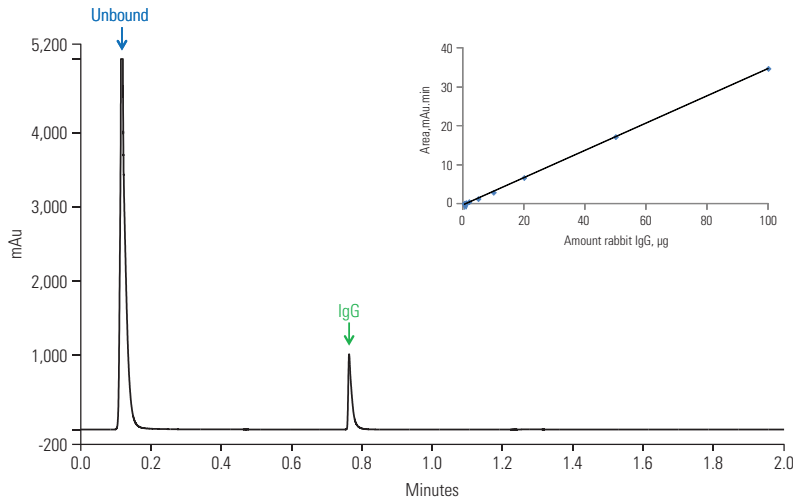
## Applications

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 is 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 proprietary hydrophilic resin, with its fast mass transfer capability, results in sharp, concentrated peaks. The HPLC compatibility of this column in combination with low back pressure and high efficiency, allows automation, providing 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.

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## Faster MAb Titer Analysis

Fast and accurate results are important in titer analysis. Figure 1, shows a 10  $\mu$ L injection of an 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). Fast mass transfer results in a fast, efficient separation. The complete cycle time, including equilibration, is 2 minutes. The MAb titer is determined using a calibration curve previously generated and the integrated IgG peak area (see insert). The unique surface chemistry provides accurate titer analysis with linearity over a wide concentration range (0.025mg/mL to 5 mg/mL).

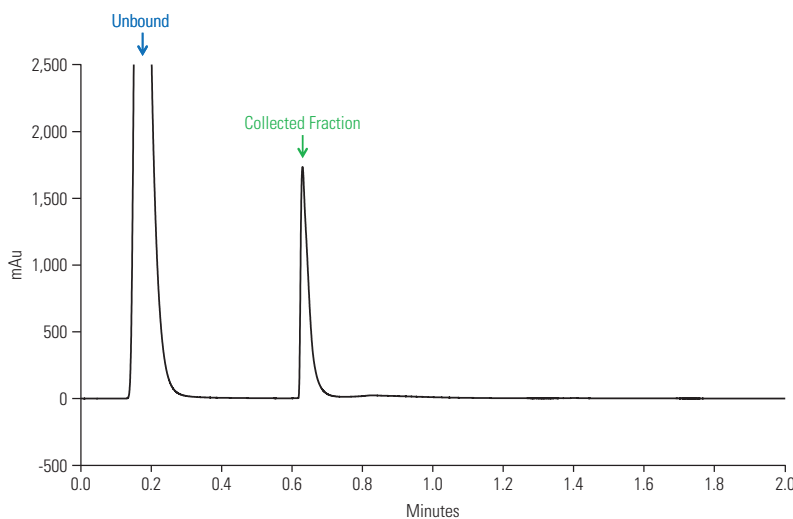


Column: **MAbPac Protein A, 4 × 35 mm**  
 Flow rate: 2 mL/min  
 Eluent A: 50 mM Sodium Phosphate, 150 mM NaCl, 5% acetonitrile, pH 7.5  
 Eluent B: 50 mM Sodium Phosphate, 150 mM NaCl, 5% acetonitrile, pH 2.5  
 Gradient: 0% B for 0.2 mins, 100% B for 0.60 mins, 0% B for 1.20 mins  
 Temperature: 25 °C  
 Detection: 280 nm  
 Injection volume: 10  $\mu$ L  
 Sample: Harvest cell culture (HCC)

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

## Purified MAb

Affinity chromatography is also a useful tool for concentrating and purifying IgG material for later experiments or 2nd 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.



Column: **MAbPac Protein A, 4 × 35 mm**  
 Flow rate: 2 mL/min  
 Eluent A: 50 mM Sodium Phosphate, 60 mM NaCl, pH 7.5  
 Eluent B: 50 mM Sodium Phosphate, 60 mM NaCl, pH 2.5  
 Gradient: 0% B for 0.2 mins, 100% B for 0.60 mins, 0% B for 1.20 mins  
 Temperature: 25 °C  
 Detection: 280 nm  
 Injection volume: 50  $\mu$ L  
 Sample: Harvest cell culture (HCC)

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

Figure 3 shows the collected Protein A purified fraction, analyzed in the second dimension on a Thermo Scientific™ MAbPac™ SCX-10, 10 µm 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 1st and the 2nd dimension analysis can be automated using the Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System providing the capability of multiple HCC samples cycled without user intervention.

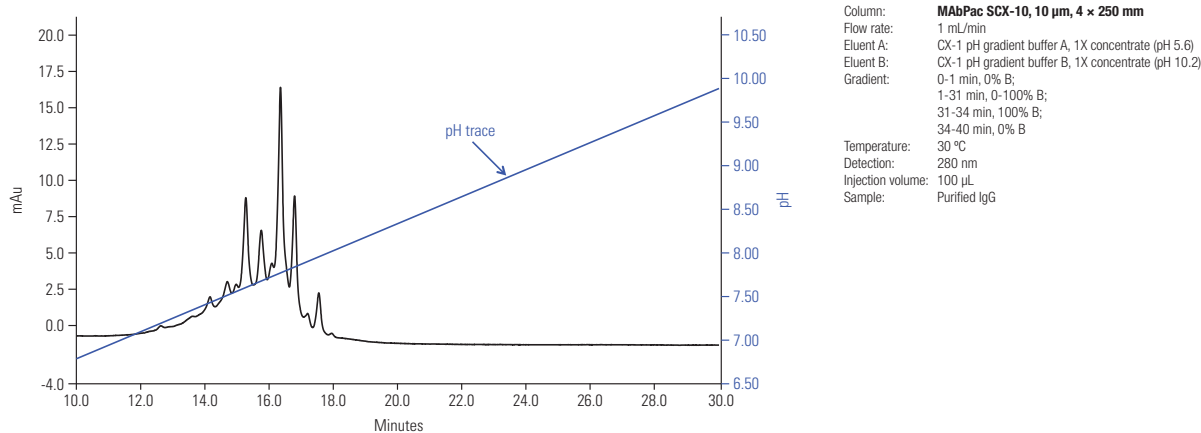


Figure 3: Charge variant analysis on the MAbPac SCX-10 column using linear pH gradient

## Reproducibility

The MAbPac Protein A column is very reproducible as shown in Figure 4. Over 2,000 injections with virtually no change in retention time, area or asymmetry. Each MAbPac Protein A column is manufactured to strict specifications to ensure column-to-column reproducibility. Each column is individually tested and shipped with a qualification assurance report.

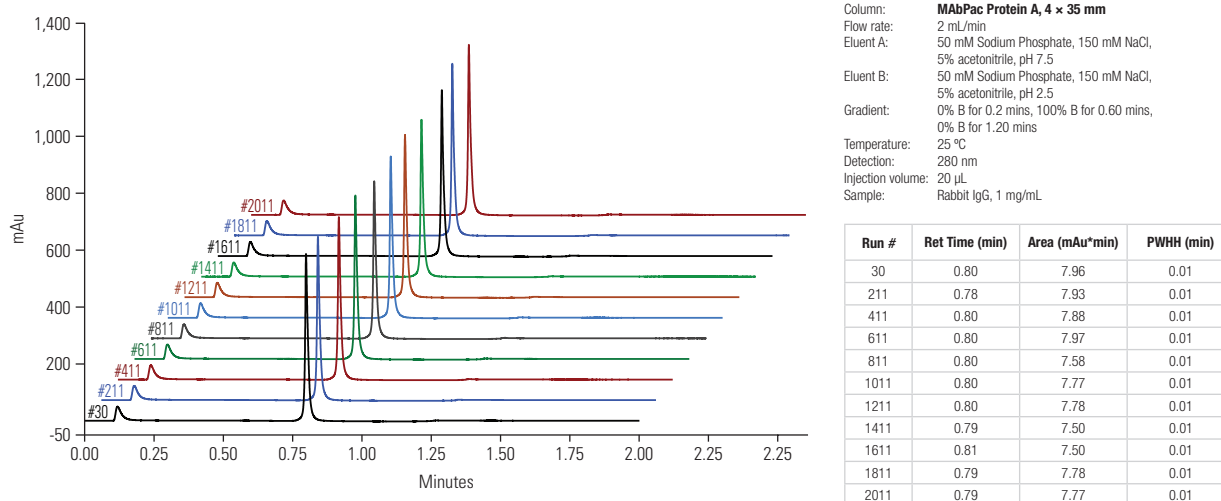


Figure 4: Reproducibility

## Physical Data

	MABPac Protein A
Substrate	Hydrophilic non-porous resin
Ligand	Protein A
Particle size	12 µm
Binding Capacity	~3.5 mg IgG/g resin
Dynamic loading Capacity	100 µg IgG/column at 2mL/min flow rate

## Specifications and Operational Parameters

Column	Particle size (µm)	Flow Rate (mL/min)	Pressure Limit (psi)	Temperature (°C)	pH Range	IgG Sample Loading (µg)
MABPac Protein A	12	≤ 2.5	≤ 1000	≤ 30	1.9–7.5	≤ 100

## Ordering Information

Description	Part Number
MabPac Protein A, Analytical column, 4 × 35 mm	082539

For more information, please visit our website at [thermoscientific.com/biolc](http://thermoscientific.com/biolc)

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