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MAbPac Protein A Column

Quickstart

Part Number 082539

1. Overview

The Thermo Scientific™ MAbPac™ Protein A column offers affinity separation for immunoglobulin (IgG). Conditioning of the column bed is required prior to initial use and after long-term storage. This QuickStart is intended to help first-time users quickly get started and also ensure extended column lifetime and reproducibility.

2. Preparation

A. Eluent Preparation:
The following eluents are recommended, but the column may be used with any eluent appropriate for analysis. Typically, **Eluent A** is a "protein-friendly" 1X PBS buffer at neutral pH and **Eluent B** is 1X PBS in acidic pH. All chemicals should be at least ACS reagent grade. All eluents should be filtered through a 0.2µm filter before use.

- **Eluent A**: 50mM Sodium Phosphate, 150 mM NaCl, pH 7.5
- **Eluent B**: 50mM Sodium Phosphate, 150 mM NaCl, pH 2.5

B. Column Installation:
Before use, allow the column to reach room temperature.
Install the column on the instrument in the correct flow direction.

WARNING

Sudden increases in flow rates may damage packed bed columns. Always increase the flow rate slowly using a linear flow gradient or stepwise increments in flow rate.
If the eluent composition generates back pressure in excess of the maximum operating pressure, reduce the flow rate to ensure the upstream back pressure is less than the maximum operating pressure.
The maximum operating pressure or flow rate limit for the MAbPac Protein A (4×35mm) is 1000 psi (6.9 MPa) or 2.5 mL/min.

3. Flow Rate Start-Up

Using a linear or stepwise flow gradient, increase the flow rate of **Eluent A** starting from 0.00 mL/min to the desired flow rate at a rate of 0.5 mL/min per minute.

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4. Column Conditioning

Use the guidelines below to determine the proper startup conditions:

A. Column Warm up:
After taking the column out of the refrigerator, let it sit on the bench at room temperature for 15 min before connecting it to the HPLC system.

B. Removal of Storage Solution:
With the column disconnected from the detector, wash the column at 2.0 mL/min with **Eluent A** for at least 5 minutes prior to column equilibration.

NOTE

Sodium Azide is highly UV absorbing and will produce a high detector response if connected to the detector.

C. Column Equilibration:
With the column connected to the detector, wash the column with **Eluent A** for at least 15 minutes.

5. Storage

A. For short-term storage, <2 days, store the column in the initial buffer compositions.

B. For long-term storage, >2 days, refrigerate the column at 2 - 8 °C in the recommended **Eluent A** with 0.1% NaN_3.

NOTE

When the column is not in use, it is recommended to store the column at 2-8 °C.

For additional information, please refer to the manual, MAbPac Protein A Product Manual Doc. No. 065505.

6. Operational Specifications

Column	Particle size (µm)	Flow Rate (mL/min)	Pressure Limit (psi)	Temperature (ºC)	pH Range	IgG Sample Loading (µg)
MAbPac Protein A	12	< 2.5	< 1000	< 30	2.5 – 7.5	< 100

7. Physical Data

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