

MABPac HIC-20 COLUMNS

Quick Start

MABPac HIC-20, 5 µm, 4.6 × 100 mm (P/N: 088553)
 MABPac HIC-20, 5 µm, 4.6 × 250 mm (P/N: 088554)
 MABPac HIC-20, 5 µm, 4.6 × 10 mm Guard Cartridges 2/pk (P/N: 088555)

1. Overview

Thermo Scientific™ MABPac™ HIC-20 is a high resolution silica based HIC column designed for separation of mAbs and related biologics. Its unique, proprietary column chemistry provides high resolution, rugged stability, and desired selectivity for the analysis of mAbs and related variants.

2. Main features of the MABPac HIC-20 Column

- Optimal selectivity for mAbs and related variants.
- Excellent separation for mAb fragments and oxidized variants.
- High resolution and high efficiency.
- Compatible with both organic solvent and aqueous mobile phase.
- Rugged column stability.

3. Specifications and Recommended Operational Parameters

Parameter	Recommendation					
Flow Rate Range	0.5-1.0 mL/min (max. 1.5 mL/min)					
Long Term Storage Solution	0.1 M ammonium acetate, pH 5.4 / acetonitrile (50:50 v/v)					
Common Mobile Phases	Mobile phase formula 1 Mobile phase A: 2 M ammonium sulfate, 100 mM sodium phosphate, pH 7.0 Mobile phase B: 100 mM sodium phosphate, pH 7.0					
	Mobile phase formula 2 Mobile phase A: 1.5 M ammonium sulfate, 50 mM sodium phosphate, pH 7.0 / isopropanol (95:5 v/v) Mobile phase B: 50 mM sodium phosphate, pH 7.0 / isopropanol (80:20 v/v)					
Solvents Compatibility	Compatible with 100% organic solvents					
Temperature Range	5 – 60 °C					
Pressure Limit	6,000 psi for 100 mm columns 8,000 psi for 250 mm columns					
pH Range	2.0-9.0					
Typical Gradient (1 mL/min flow rate)	4.6 × 100 mm		4.6 × 250 mm			
	Time (min)	%A	%B	Time (min)	%A	%B
	-5.0	100	0	-10.0	100	0
	0.0	100	0	0.0	100	0
	1.0	100	0	1.0	100	0
	15.0	0	100	36.0	0	100
20.0	0	100	42.0	0	100	

Note: Inject sample at 0 min.

4. Operational Guidelines

- Must use and store the column in buffered conditions.
- Operate the column within operating parameters and specifications (described in Section 3).
- Column conditioning: when used for the first time or after stored in the long term storage solution, it is recommended to condition the column by injecting high concentrations (e.g. 10 mg/mL) of ovalbumin, BSA, or the protein of your choice. Inject ≥ 750 μg of protein using the gradient specified in Section 3.
- Avoid any sudden pressure surge. Watch the flow setting on the pump before connecting to the column.
- When switching from aqueous to organic phase, or organic to aqueous phase, make sure that the flow rate is equal to or less than 0.2 mL/min.
- When not in use, stop the flow and store the column in the low salt concentration mobile phase for short period of time (overnight) and in 0.1 M ammonium acetate, pH 5.4 / acetonitrile (50:50 v/v) for long term.
- Use a guard column when injecting crude protein samples; to protect the analytical column and to extend column lifetime. Note that the guard cartridge requires the cartridge holder (P/N 069580). Dirty, particulate samples should be cleaned with a 0.2 μm filter before applying onto the column.
- Sample preparation: To ensure proper binding of the sample, dissolve the sample in starting mobile phase.
 - If the sample is solid, dissolve the sample in the starting mobile phase. If the sample precipitates at this salt concentration, dissolve the sample in water or appropriate buffer then dilute the sample in half with the starting mobile phase.
 - If your sample is already in a solution, simply dilute the sample in half with the starting mobile phase.
 - *Note: Make sure the sample does not precipitate.*
- Column washing: Wash the column with 0.05% acetic acid for 30 column volumes at 0.2 mL/min → Wash the column with isopropanol for 30 column volumes at 0.2 mL/min → Wash the column with 0.05% acetic acid again for 10 column volumes at 0.2 mL/min.
- For method optimization refer to Section 3 of the manual.

5. Ordering Information

	Particle Size	Column Dimensions	P/N	Required Holder
Analytical Column	5 μm	4.6 x 100 mm	088553	
		4.6 x 250 mm	088554	
Guard	5 μm	4.6 x 10 mm (2/pk)	088555	P/N 069580