

# Polar MC HIC Resins

## Hydrophobic Interaction Chromatography for Biomolecule Purification

### Polar MC-HIC

#### Introduction

Hydrophobic Interaction Chromatographic (HIC) separation applies to all stages of biological sample purification process including capturing and polishing. Sepax manufactures two HIC products: Generik MC-HIC and Polar MC-HIC. Both Polar MC-HIC and Generik MC-HIC are made of polymethacrylate matrix, **while Generik MC is more hydrophobic than Polar MC.**

#### Product Description

Polar MC30-HIC chromatographic media is composed of hydrophilic polymethacrylate beads functionalized with ether or butyl on the surface, as shown in Figure 1, which provide appropriate hydrophobic characteristics and interactions with biological analytes. The resin has an average particle size of 30  $\mu\text{m}$  and pore size of 800  $\text{\AA}$ . Due to its high physical and chemical stability, it can tolerate high-pressure operation up to 100 bars which allows faster flow rate and shorter running time. Polar MC30-HIC is designed for various stages of hydrophobic interaction chromatography separation needs, from laboratory discovery, pilot-scale purification to industrial process.

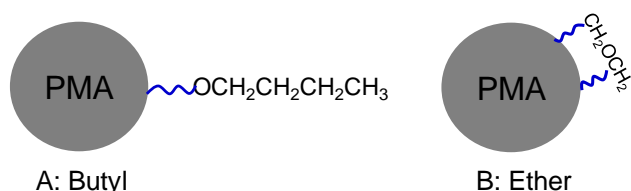


Figure 1. Structures of Polar MC30-HIC Butyl and Ether

#### Characteristics

- Unique hydrophobic interaction
- High accessible surface area and loading capacity
- High chemical stability for low leaching
- High physical stability for high-pressure operation
- Capable of running high flow rate to speed up separation
- Spherical particles with high mechanical strength
- Wide pH tolerance (pH=2-14)
- Available from 1 L to >100 L

#### Technical Specifications

Media Type	Polar MC30-HIC Butyl Polar MC30-HIC Ether
Packing	70% (v/v) slurry in 20% ethanol
Matrix	Hydrophilic polymethacrylate
Particle Size	20 - 45 $\mu\text{m}$ (average 30 $\mu\text{m}$ )
Pore Structure	800 $\text{\AA}$
Dynamic Binding Capacity*	15 $\pm$ 5 mg Lysozyme /mL for Polar MC-HIC Ether 35 $\pm$ 5 mg Lysozyme /mL for Polar MC-HIC Butyl
pH Stability	2-14
Operating Temperature	Up to 40 $^{\circ}\text{C}$
Resin pressure limit	Up to 100 bar
Mobile Phase Compatibility	Compatible with aqueous solution, a mixture of water and acetonitrile, acetone, or methanol. Typical buffers: phosphate, Tris, and acetate.
Linear Flow Rate	Up to 7200 cm/hour

\*DBC was tested with lysozyme (1 mg/mL) in 25 mM sodium phosphate pH 7 + 2 M  $(\text{NH}_4)_2\text{SO}_4$  with 360 cm/h flow rate at 280 nm, 10% breakthrough.

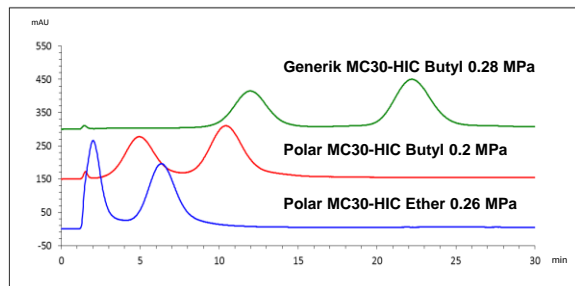
#### Applications

Polar MC30-HIC media offers excellent high efficiency and recovery separation of bio-molecules such as mAb (monoclonal antibody), ADC (antibody drug conjugate) and related protein fragments, DNA and oligonucleotides.



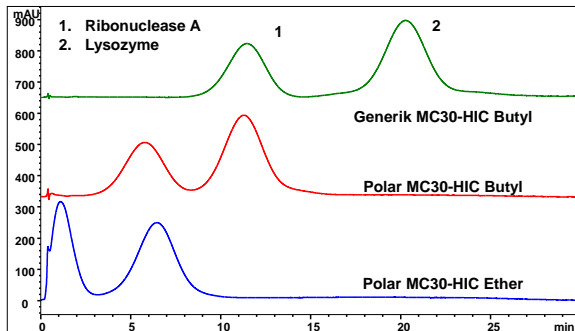
## Phase Selectivity

Figure 2. Generik & Polar MC30-HIC Comparison on the Analysis of Ribonuclease A and lysozyme- Glass Column



Column: Polar MC30-HIC Ether/Polar M30C-HIC Butyl/Generik MC30-HIC Butyl (bed volume 5 ml, Generik FPLC Empty Glass Column 10x150mm; P/N: 202000-1015-AF)  
 Mobile Phase A: 25 mM Sodium phosphate + 2M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, pH 7.0  
 Mobile Phase B: 25 mM Sodium phosphate, pH 7.0  
 Injection: 100 µL  
 Flow Rate: 4 mL/min  
 Detection: FPLC, UV 214 nm  
 Gradient: 0-30 min 0-100%B  
 Sample: Ribonuclease A, Lysozyme (5 mg/mL in water)

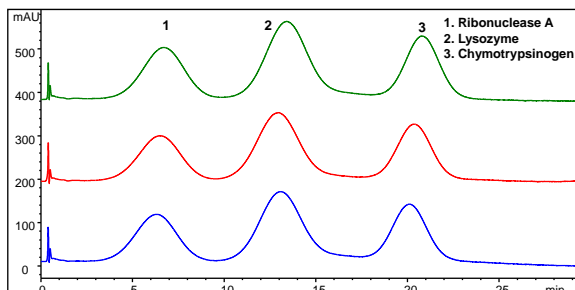
Figure 3. Generik & Polar MC30-HIC Comparison on the Analysis of Ribonuclease A and lysozyme – Stainless Steel Column



Column: Polar MC30-HIC Ether/Polar MC30-HIC Butyl/Generik MC30-HIC Butyl (4.6 x 50mm)  
 Mobile Phase A: 25 mM Sodium phosphate + 2M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, pH 7.0  
 Mobile Phase B: 25 mM Sodium phosphate, pH 7.0  
 Injection: 20 µL  
 Flow Rate: 1.5 mL/min  
 Detection: UV 214 nm  
 Gradient: 0-30 min 0-100%B  
 Sample: Ribonuclease A, Lysozyme (5 mg/mL in water)

## Lot to Lot Consistency

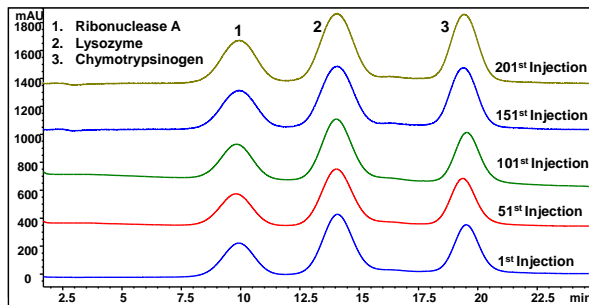
Figure 4. Polar MC30-HIC Butyl Lot to Lot Consistency



Column: Polar MC30-HIC Butyl (4.6 x 50mm)  
 Mobile Phase A: 25 mM Sodium phosphate + 2M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, pH 7.0  
 Mobile Phase B: 25 mM Sodium phosphate pH 7.0  
 Injection: 20 µL  
 Flow Rate: 1.5 mL/min  
 Detection: UV 214 nm  
 Gradient: 0-30 min 0-100%B  
 Sample: Ribonuclease A, Lysozyme, Chymotrypsinogen (2 mg/mL in water)

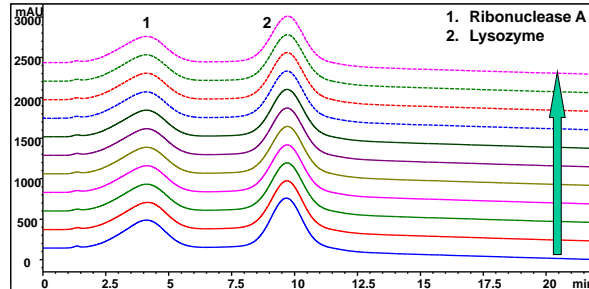
## Life Time

Figure 5. Polar MC30-HIC Butyl Lifetime Test



Column: Polar MC30-HIC Butyl (4.6 x 50mm)  
 Mobile Phase A: 25 mM Sodium phosphate + 2M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, pH 7.0  
 Mobile Phase B: 25 mM Sodium phosphate, pH 7.0  
 Injection: 10 µL  
 Flow Rate: 0.5 mL/min  
 Detection: UV 214 nm  
 Gradient: 0-22 min 0-100%B  
 Sample: Ribonuclease A, Lysozyme, Chymotrypsinogen (2 mg/mL in water)

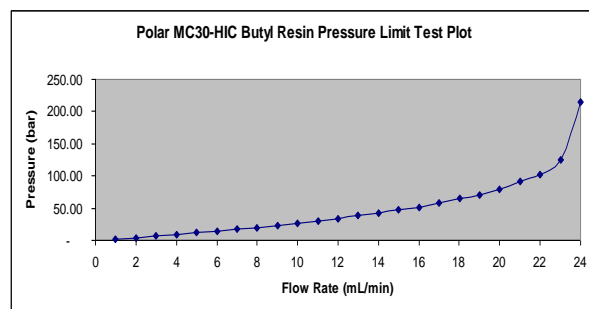
Figure 6. Polar MC30-HIC Butyl CIP Test 10 Cycles



Column: Polar MC30-HIC Butyl (4.6 x 50mm)  
 CIP: 1 M NaOH, Flow rate: 0.2 mL/min, 15 min.  
 Wash: 25mM Sodium phosphate + 2M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, pH 7.0, Flow rate: 1.5 mL/min, 10min.  
 QC Mobile Phase A: 25 mM Sodium phosphate + 2 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, pH 7.0  
 QC Mobile Phase B: 25 mM Sodium phosphate, pH 7.0  
 Injection: 20 µL  
 Flow Rate: 1.5 mL/min  
 Detection: UV 214 nm  
 Gradient: 0-30 min 0-100%B  
 Sample: Ribonuclease A, Lysozyme, Chymotrypsinogen (2 mg/mL in water)

## Pressure Limit

Figure 7. Polar MC30-HIC Butyl Pressure Limit Test

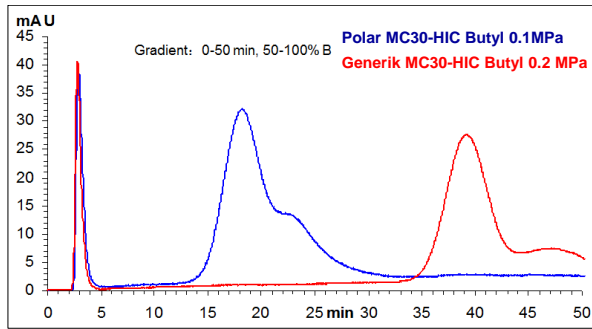


Column: Polar MC30-HIC Butyl (4.6 x 150mm)  
 Mobile Phase: 25 mM Sodium phosphate + 2.0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, pH 7.0  
 Flow rate: 1-24 mL/min  
 Instrument: Waters HPLC

## Applications

### *mAb analysis on Generik & Polar MC30-HIC Butyl*

Figure 8. MAb Analysis on Generik& Polar MC30-HIC Butyl



Column: Polar MC30-HIC Butyl/Generik MC30-HIC Butyl  
(Bed volume, 5 mL, Generik FPLC Empty Glass Column  
10 x150 mm; P/N: 202000-1015-AF)  
Mobile Phase A: 25 mM Sodium phosphate + 2.0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, pH 7.0  
Mobile Phase B: 25 mM Sodium phosphate, pH 7.0  
Injection: 50 µL  
Flow Rate: 2.0 mL/min  
Detection: FPLC, UV 214 nm  
Gradient: 0-50 min 50-100%B  
Sample: MAb (2.5 mg/mL in water)

## Ordering Information

### Polar MC30-HIC Media

Description	Particle size	Resin volume	P/N
Polar MC-HIC Butyl	30 µm	100 mL	258030-0000
Polar MC-HIC Butyl	30 µm	1 L	258030-0000
Polar MC-HIC Butyl	30 µm	5 L	258030-0000
Polar MC-HIC Butyl	30 µm	20L	258030-0000
Polar MC-HIC Ether	30 µm	100 mL	258130-0000
Polar MC-HIC Ether	30 µm	1 L	258130-0000
Polar MC-HIC Ether	30 µm	5 L	258130-0000
Polar MC-HIC Ether	30 µm	20L	258130-0000

\*Different package sizes also available

### Polar MC30 HIC Column

Description	Dimension	P/N
HIC 30um Screening Kit, 3 different HIC phases	4.6 x 50 mm	HIC30S-4605 <sup>[1]</sup>
HIC 30um Screening Kit, 3 different HIC phases	7 x 25 mm	HIC30P-70025 <sup>[2]</sup>
Polar MC-HIC Butyl	7 x 25 mm	258030-70025 <sup>[2]</sup>
Polar MC-HIC Butyl	16 x 25 mm	258030-160025 <sup>[2]</sup>
Polar MC-HIC Butyl	7.8 x 100 mm	258030-7810 <sup>[1]</sup>
Polar MC-HIC Butyl	21.2 x 100 mm	258030-21210 <sup>[1]</sup>
Polar MC-HIC Ether	7 x 25 mm	258130-70025 <sup>[2]</sup>
Polar MC-HIC Ether	16 x 25 mm	258130-160025 <sup>[2]</sup>
Polar MC-HIC Ether	7.8 x 100 mm	258130-7810 <sup>[1]</sup>
Polar MC-HIC Ether	21.2 x 100 mm	258130-21210 <sup>[1]</sup>

[1] Stainless Steel Tubing

[2] Plastic Cartridge

### Related Product- Generik<sup>®</sup> FPLC Empty Column

P/N	End-fitting	Column Size (mm)	Bed Height (cm)	Volume (ml)
202000-0615-FF	F/F	6.6 x 150	12	4.1
202000-0615-AF	A/F	6.6 x 150	4-12	1.4-4.1
202000-0625-AA	A/A	6.6 x 250	6-22	2.1-7.5
202000-0625-AF	A/F	6.6 x 250	14-22	4.8-7.5
202000-0640-AA	A/A	6.6 x 400	21-37	7.2-12.7
202000-1015-AF	A/F	10.0 x 150	4-12	3.1-9.4
202000-1025-AA	A/A	10.0 x 250	6-22	3.1-17.3
202000-1025-AF	A/F	10.0 x 250	14-22	11-17.3
202000-1515-AF	A/F	15.0 x 150	4-12	7.1-21.2
202000-1525-AA	A/A	15.0 x 250	6-22	10.6-38.9
202000-1525-AF	A/F	15.0 x 250	14-22	24.7-38.9
202000-2515-AF	A/F	25.0 x 150	4-12	19.6-58.9
202000-2525-AA	A/A	25.0 x 250	6-22	29.4-108.0
202000-2525-AF	A/F	25.0 x 250	14-22	68.7-108.0
202000-3540-AF	A/F	35.0 x 400	29-37	279.0-355.9
202000-5015-AF	A/F	50.0 x 150	4-12	77.5-235.0
202000-5040-AA	A/A	50.0 x 400	21-37	420.5-741.0
202000-5040-AF	A/F	50.0 x 400	29-37	580.7-741.0
202000-5050-AA	A/A	50.0 x 500	31-47	620.8-941.2
202000-5050-AF	A/F	50.0 x 500	39-47	781.0-941.2

\*AF: One fixed endpiece and one adjustable endpiece

AA: Two adjustable endpieces

FF: Non-adjustable with two fixed endpieces

\*Please visit our website for the most updated literature