Generik MC HIC Resins

Hydrophobic Interaction Chromatography for Biomolecule Purification

Generik MC-HIC Butyl

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

Generik MC30-HIC Butyl media is composed of polymethacrylate beads functionalized with butyl on the surface, as shown in Figure 1. The resin has an average particle size of 30 µm and pore size of 800 Å. 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. Generik MC-30 HIC is designed for various stages of hydrophobic interaction chromatography separation needs, from laboratory discovery, pilot-scale purification to industrial process.

Figure 1. *Structure of Generik MC30-HIC Butyl*

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	Generik MC30-HIC Butyl
Packing	70% (v/v) slurry in 20% ethanol
Matrix	Polymethacrylate
Particle Size	20 - 45 µm (average 30 µm)
Pore Structure	800 Å
Dynamic Binding Capacity*	45 ± 5 mg Lysozyme/mL
pH Stability	2-14
Operating Temperature	Up to 40 °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

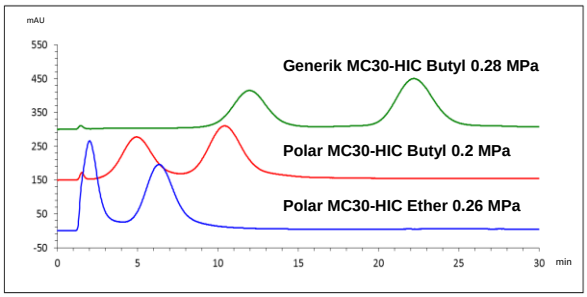
*DBC was tested with lysozyme (1 mg/mL) in 25 mM sodium phosphate pH 7 + 2 M $(NH_4)_2SO_4$ with 360 cm/h flow rate at 280 nm, 10% breakthrough.

Applications

Generik MC30-HIC Butyl 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.

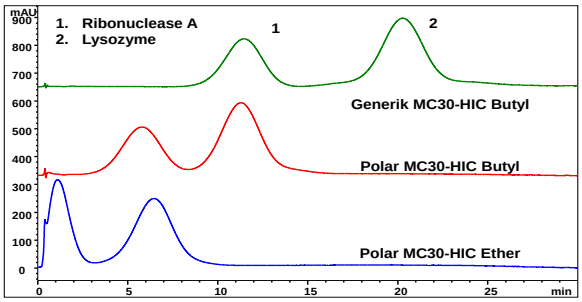
Phase Selectivity

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 $(NH_4)_2SO_4$, 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)

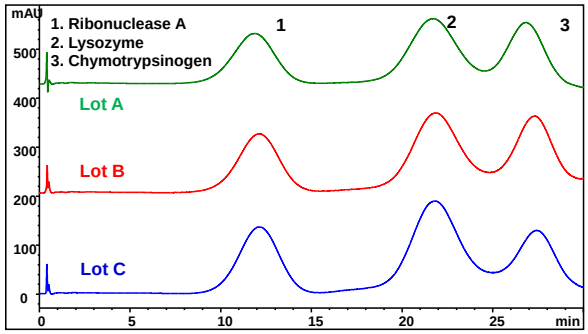
Figure 3.Generik& Polar MC30-HIC Comparison on the Analysis of Ribonuclease A and lysozyme – Stainless Steel Column



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 $(NH_4)_2SO_4$, 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

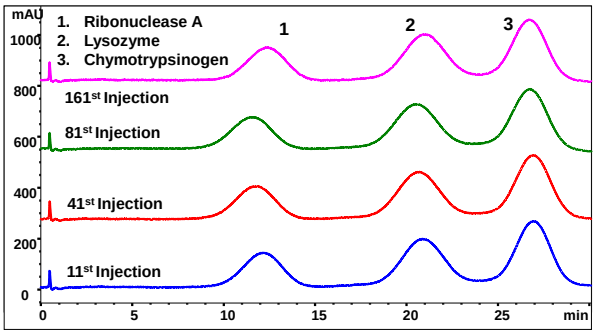
Figure 4. Generik MC30-HIC Butyl Lot to Lot Consistency



Column:	Generik MC30-HIC Butyl (4.6 x 50mm)
Mobile Phase A:	25 mM Sodium phosphate + 2M $(NH_4)_2SO_4$, 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)

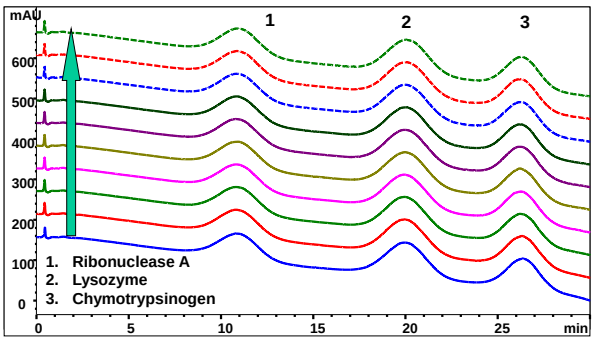
Life Time

Figure 5.Generik MC30-HIC Butyl Lifetime Test



Column:	Generik MC30-HIC Butyl (4.6 x 50mm)
Mobile Phase A:	25 mM Sodium phosphate + 2M $(NH_4)_2SO_4$, pH 7.0
Mobile Phase B:	25 mM Sodium phosphate, pH 7.0
Injection:	10 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)

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

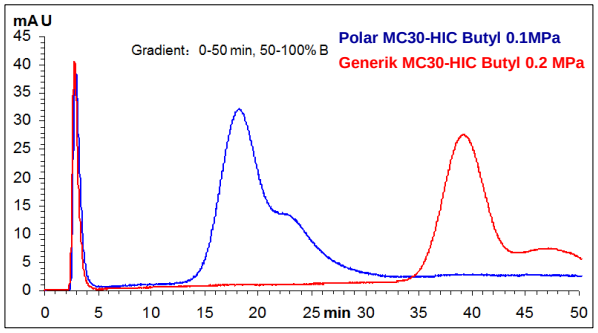


Column:	Generik MC30-HIC Butyl (4.6 x 50mm)
CIP:	1 M NaOH, Flow rate: 0.2 mL/min, 15 min.
Wash:	25mM Sodium phosphate + 2M $(NH_4)_2SO_4$, pH 7.0, Flow rate: 1.5 mL/min, 10min.
QC Mobile Phase A:	25 mM Sodium phosphate + 2 M $(NH_4)_2SO_4$, pH 7.0
QC Mobile Phase B:	25 mM Sodium phosphate, pH 7.0
Injection:	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)

Applications

mAb analysis on Generik & Polar MC30-HIC Butyl

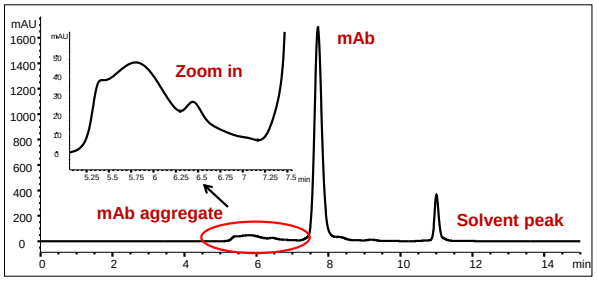
Figure 7. Generik & Polar MC30-HIC Butyl comparison on the analysis of mAb – Glass Column



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 $(NH_4)_2SO_4$, 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)

Before HIC Purification

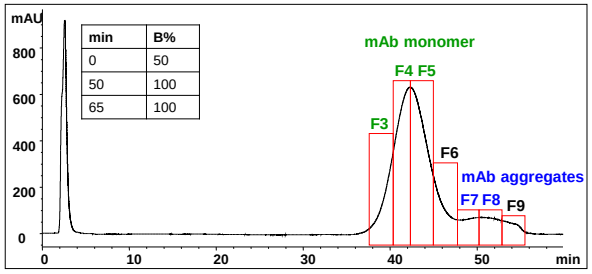
Figure 8. Semi-purified mAb Analysis on Zenix SEC-300



Column:	Zenix SEC-300 (3 µm, 7.8 x 300 mm, PN: 213300-7830)
Mobile Phase:	150 mM sodium phosphate buffer (pH7.0)
Injection:	5 µL
Flow Rate:	1 mL/min
Detection:	UV 214 nm
Sample:	mAb (5 mg/mL)

HIC Purification

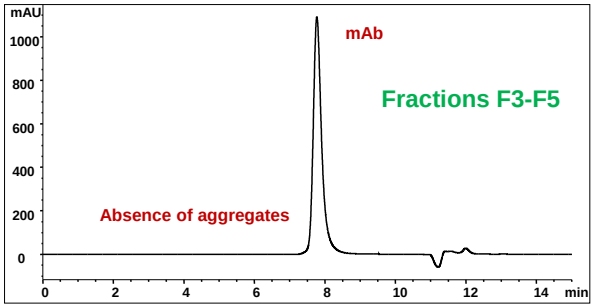
Figure 9. mAb Purification on Generic MC 30-HIC Butyl



Column:	Generic MC 30-HIC Butyl (1 mL, plastic cartridge, PN:248030-70025)
Mobile Phase A:	20 mM Na_2HPO_4 , 1.7 M $(NH_4)_2SO_4$, pH 7.0
Mobile Phase B:	20 mM Na_2HPO_4 , pH 7.0
Injection:	50 µL
Flow Rate:	0.4 mL/min
Pressure:	13.7 bar
Detection:	UV 214 nm
Gradient:	0-50 min 50-100%B
Sample:	mAb (5 mg/mL)

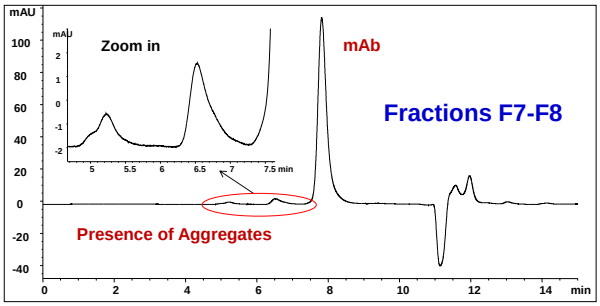
Post HIC Purification – SEC Confirmation

Figure 10. mAb Fraction Analysis on Zenix SEC-300



Column:	Zenix SEC-300 (3 µm, 7.8 x 300 mm, PN: 213300-7830)
Mobile Phase:	150 mM sodium phosphate buffer (pH7.0)
Injection:	100 µL
Flow Rate:	1 mL/min
Detection:	UV 214 nm
Sample:	Fractions F3-F5

Figure 11. mAb Fraction Analysis on Zenix SEC-300



Column:	Zenix SEC-300 (3 µm, 7.8 x 300 mm, PN: 213300-7830)
Mobile Phase:	150 mM sodium phosphate buffer (pH7.0)
Injection:	100 µL
Flow Rate:	1 mL/min
Detection:	UV 214 nm
Sample:	Fractions F7 and F8

Ordering Information

Generik MC30-HIC Media

Description	Particle size	Resin volume	P/N
Generik MC-HIC Butyl	30 µm	100 mL	248030-0000
Generik MC-HIC Butyl	30 µm	1 L	248030-0000
Generik MC-HIC Butyl	30 µm	5 L	248030-0000
Generik MC-HIC Butyl	30 µm	20L	248030-0000

Generik MC30 HIC Column

Description	Dimension	P/N
HIC 30um Screening Kit, 3 different HIC phases	4.6 x 50 mm	HIC30S-4605[1]
HIC 30um Screening Kit, 3 different HIC phases	7 x 25 mm	HIC30P-70025[2]
Generik MC-HIC Butyl	7 x 25 mm	248030-70025[2]
Generik MC-HIC Butyl	16 x 25 mm	248030-160025[2]
Generik MC-HIC Butyl	7.8 x 100 mm	248030-7810[1]
Generik MC-HIC Butyl	21.2 x 100 mm	248030-21210[1]

[1] Stainless Steel Tubing
[2] Plastic Cartridge

Related Product- Generik® 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 literatue