Data sheet

Butyl (4HF, 6HF, Big Beads)

Phenyl (Super, 4HF, 6HF, Big Beads)

Octyl (4HF, 6HF, Big Beads)

1. Introduction

Butyl, Phenyl and Octyl Agarose Resins are hydrophobic interaction chromatography (HIC) adsorbents having a carbon chain of C4, benzene ring and C8, respectively. They are specially designed for the purification of biological molecules based on their hydrophobicity profiles.

HIC is a versatile technique and could show high selectivity to individual molecules according to their exposed hydrophobic zones. It is particularly useful for intermediate and final-stage purifications. A HIC medium normally binds at moderate to high salt concentrations. It is logical to place HIC step after an IEX step where molecules are usually eluted at high salt conditions.

HIC media shows much milder purification conditions than reversed phase chromatography (RPC) media. Better biological activity could be maintained in HIC operations than RPC operations.

Protein Ark's HIC media offers a broad range of choices. Generally speaking, the longer the carbon chain, the higher the surface hydrophobicity. The base matrix is made of agarose that has been highly cross-linked. It is very stable to most of the chemical conditions experienced in bioprocessing industry. Process developers and manufacturers have the chance to choose the best HIC media for their applications. The core advantages are:

- High sample loading capacity
- More choices than other suppliers
- High separation power
- All versions can be scaled up and produced for GMP use
- Regulatory support files available

The feature and selection guide are listed below:

Fastback 4HF (50 – 150 μm)	**Fastback 6HF** (50 – 150 μm)	**Big Bead** (150 – 350 μm)	**Super** (25 – 50 μm)
The above HIC media is designed to purify most medium to large proteins	The above HIC media is designed to purify peptides or smaller proteins	The above HIC media is designed to purify proteins from crude or viscous samples	The above HIC media is designed to purify proteins that require high resolution

Characteristics of Protein Ark's HIC media:

	Butyl	Phenyl	Octyl
Matrix	Highly cross-linked agarose; 4% agarose for fastback 4HF, 6% agarose for fastback 6HF		
Functional group	$-(CH_2)_3CH_3$	$-C_6H_6$	$-(CH_2)_7CH_3$
Ligand density	Around 40 μmol/ml	Around 25 μmol/ml Around 40 μmol/ml (high sub)	Around 4 μmol/ml
Binding capacity	>20 mg lyzosyme / ml	>20 mg BSA / ml	>20 mg BSA / ml
Particle size	25 - 50 μm (Super), 50 - 150 μm (4HF and 6HF), 150 - 350 μm (Big Bead)		
Pressure-flow property*	>100 cm/h for Super; >300 cm/h for 4HF; >500 cm/h for 6HF; >1500 cm/h for Big Bead		
Operational pressure	Up to 3 bar		
pH stability	2-14 (short term) and 3-12 (long term)		
Working temperature	$+4^{O}C$ to $+30^{O}C$		
Chemical stability	All commonly used buffers; 1 M acetic acid, 1 M NaOH, 6M guanidine hydrochloride, 8 M urea, 30% acetonitrile, 30% isopropanol, 70% ethanol, 3 M $(NH_4)_2SO_4$		
Storage	20% ethanol		

**Measured in a 32 mm ID column at a bed height of 15 cm.*

2. Method optimization

We recommend scouting the parameters among loading capacity, flow velocity, binding pH, binding ionic strength, elution speed and gradient etc. We recommend paying special attention to optimize elution conditions to achieve the best separation power.

In general, balancing the degree of component separation against process throughput is the major consideration when optimizing a method. Besides, for the purification of instable or shearing-force sensitive molecules, the operational condition needs be optimised to balance the throughput and the possible damage to the target molecule.

3. Maintenance

Depending on the individual applications, the media may be used many times. For the re-use purpose, please see the following instructions.

Cleaning-in-place (CIP)

CIP is a procedure that removes strongly bound materials such as lipids, endotoxins and denatured proteins that remain in the adsorbent surface after the regeneration. Regular CIP prevents the build up of contaminants in the packed bed and helps to maintain the column performance.

A specific CIP protocol should be developed for each process according to the type of contaminants present. The frequency of CIP depends on the nature of individual applications.

The following information works as a general guidance.

The contaminants bound by hydrophobic nature can be removed by the following reagents: 1 M NaOH, low percentage non-ionic detergents (e.g. 0.1 – 2%), 30% isopropanol in basic or acidic conditions (e.g. in the presence of acetic acid or phosphoric acid). A combination of the above reagents can be explored as well. In general, the incubation time should be longer (e.g. from 30 minutes to 2 hours) to ensure full dissociation of the contaminants.

Sanitization
Sanitization using 0.5-1.0 M NaOH with a contact time of 30 mins is recommended.

4. Storage

The media should be stored in 20% ethanol or 0.02% sodium azide to prevent microbial growth. Store the media at a temperature of +4°C to +30°C. Before use, equilibrate the media with at least 5 bed volumes of the running buffer.

5. Ordering information

Product Description	Pack Size	Product Code
Fastback Butyl 4 High Flow Resin	25 ml	Fastback-Butyl-4HF-25
	100 ml	Fastback-Butyl-4HF-100
	250 ml	Fastback-Butyl-4HF-250
	500 ml	Fastback-Butyl-4HF-500
	1 litre	Fastback-Butyl-4HF-1L
	5 litre	Fastback-Butyl-4HF-5L
	10 litre	Fastback-Butyl-4HF-10L
Fastback Butyl 6 High Flow Resin	25 ml	Fastback-Butyl-6HF-25
	100 ml	Fastback-Butyl-6HF-100
	250 ml	Fastback-Butyl-6HF-250
	500 ml	Fastback-Butyl-6HF-500
	1 litre	Fastback-Butyl-6HF-1L
	5 litre	Fastback-Butyl-6HF-5L
	10 litre	Fastback-Butyl-6HF-10L
Fastback Butyl Big Bead Resin	25 ml	Fastback-Butyl-BB-25
	100 ml	Fastback-Butyl-BB-100
	250 ml	Fastback-Butyl-BB-250
	500 ml	Fastback-Butyl-BB-500
	1 litre	Fastback-Butyl-BB-1L
	5 litre	Fastback-Butyl-BB-5L
	10 litre	Fastback-Butyl-BB-10L
Fastback Phenyl 4 High Flow Resin	25 ml	Fastback-Phenyl-4HF-25
	100 ml	Fastback-Phenyl-4HF-100
	250 ml	Fastback-Phenyl-4HF-250
	500 ml	Fastback-Phenyl-4HF-500
	1 litre	Fastback-Phenyl-4HF-1L
	5 litre	Fastback-Phenyl-4HF-5L
	10 litre	Fastback-Phenyl-4HF-10L
Fastback Phenyl 6 High Flow Resin	25 ml	Fastback-Phenyl-6HF-25
	100 ml	Fastback-Phenyl-6HF-100
	250 ml	Fastback-Phenyl-6HF-250
	500 ml	Fastback-Phenyl-6HF-500
	1 litre	Fastback-Phenyl-6HF-1L
	5 litre	Fastback-Phenyl-6HF-5L
	10 litre	Fastback-Phenyl-6HF-10L

Fastback Phenyl 6 High Flow Resin, HS	25 ml 100 ml 250 ml 500 ml 1 litre 5 litre 10 litre	Fastback-Phenyl-6HFHS-25 Fastback-Phenyl-6HFHS-100 Fastback-Phenyl-6HFHS-250 Fastback-Phenyl-6HFHS-500 Fastback-Phenyl-6HFHS-1L Fastback-Phenyl-6HFHS-5L Fastback-Phenyl-6HFHS-10L
Super Phenyl Resin	25 ml 100 ml 250 ml 500 ml 1 litre 5 litre 10 litre	Super-Phenyl-25 Super-Phenyl-100 Super-Phenyl-250 Super-Phenyl-500 Super-Phenyl-1L Super-Phenyl-5L Super-Phenyl-10L
Fastback Phenyl Big Bead Resin	25 ml 100 ml 250 ml 500 ml 1 litre 5 litre 10 litre	Fastback-Phenyl-BB-25 Fastback-Phenyl-BB-100 Fastback-Phenyl-BB-250 Fastback-Phenyl-BB-500 Fastback-Phenyl-BB-1L Fastback-Phenyl-BB-5L Fastback-Phenyl-BB-10L
Fastback Octyl 4 High Flow Resin	25 ml 100 ml 250 ml 500 ml 1 litre 5 litre 10 litre	Fastback-Octyl-4HF-25 Fastback-Octyl-4HF-100 Fastback-Octyl-4HF-250 Fastback-Octyl-4HF-500 Fastback-Octyl-4HF-1L Fastback-Octyl-4HF-5L Fastback-Octyl-4HF-10L
Fastback Octyl 6 High Flow Resin	25 ml 100 ml 250 ml 500 ml 1 litre 5 litre 10 litre	Fastback-Octyl-6HF-25 Fastback-Octyl-6HF-100 Fastback-Octyl-6HF-250 Fastback-Octyl-6HF-500 Fastback-Octyl-6HF-1L Fastback-Octyl-6HF-5L Fastback-Octyl-6HF-10L
Fastback Octyl Big Bead Resin	25 ml 100 ml 250 ml 500 ml 1 litre 5 litre 10 litre	Fastback-Octyl-BB-25 Fastback-Octyl-BB-100 Fastback-Octyl-BB-250 Fastback-Octyl-BB-500 Fastback-Octyl-BB-1L Fastback-Octyl-BB-5L Fastback-Octyl-BB-10L

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