

Data sheet

SepFast™ Butyl (400, 500, 600)
SepFast™ Pentyl (400, 500, 600)
SepFast™ Hexyl (400, 500, 600)
SepFast™ Phenyl (400, 500, 600)
SepFast™ Heptyl (400, 500, 600)
SepFast™ Octyl (400, 500, 600)

1. Introduction

SepFast Butyl, SepFast Pentyl, SepFast Hexyl, SepFast Phenyl, SepFast Heptyl and SepFast Octyl are hydrophobic interaction chromatography (HIC) adsorbents having a carbon chain of C4, C5, C6, C6 ring, C7 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.

SepFast HIC media offers a broad range of choice from C4 to C8. Generally speaking, the longer the carbon chain, the higher the surface hydrophobicity. Researchers have the chance to choose the best HIC medium for their applications. The core advantages are:

- High sample loading capacity
- More choices than other suppliers
- High separation power

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 the bioprocessing industry.

For each type of HIC ligand, there is a choice of 3 different base matrices according to the pore accessibility of target molecules. The feature and selection guide is listed as follows:

| 400 serial | 500 serial | 600 serial |
|--|--|---|
| SepFast Butyl-400 | SepFast Butyl-500 | SepFast Butyl-600 |
| SepFast Pentyl-400 | SepFast Pentyl-500 | SepFast Pentyl-600 |
| SepFast Hexyl-400 | SepFast Hexyl-500 | SepFast Hexyl-600 |
| SepFast Phenyl-400 | SepFast Phenyl-500 | SepFast Phenyl-600 |
| SepFast Heptyl-400 | SepFast Heptyl-500 | SepFast Heptyl-600 |
| SepFast Octyl-400 | SepFast Octyl-500 | SepFast Octyl-600 |
| The above HIC media is designed to purify peptides or small proteins | The above HIC media is designed to purify most medium to large proteins. | The above HIC media is designed to purify large to very large proteins. |

Characteristics of SepFast HIC media:

| | Butyl-400 | Pentyl-400 | Hexyl-400 | Phenyl-400 | Heptyl-400 | Octyl-400 |
|-------------------------|---|--|--|--------------------------------|--|--|
| | Butyl-500 | Pentyl-500 | Hexyl-500 | Phenyl-500 | Heptyl-500 | Octyl-500 |
| | Butyl-600 | Pentyl-600 | Hexyl-600 | Phenyl-600 | Heptyl-600 | Octyl-600 |
| Matrix | Highly cross-linked agarose | | | | | |
| Functional group | -(CH ₂) ₃ CH ₃ | -(CH ₂) ₄ CH ₃ | -(CH ₂) ₅ CH ₃ | -C ₆ H ₆ | -(CH ₂) ₆ CH ₃ | -(CH ₂) ₇ CH ₃ |
| Particle size | 50 - 150 μm | | | | | |
| Pressure-flow property* | >1000 cm/h for 400 serials; >500 cm/h for 500 serials; >300 cm/h for 600 serials | | | | | |
| Operational pressure | Up to 3 bar | | | | | |
| pH stability | 2-14 (short term) and 3-12 (long term) | | | | | |
| Working temperature | +4°C to +30°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) ₂ SO ₄ | | | | | |
| Storage | 20% ethanol | | | | | |

*Measured in a 32 mm ID column at a bed height of 20 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 to pay 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 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 1 hour 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 volume of running buffer.

5. Ordering information

| Product | Quantity | Code no. |
|--------------------------------------|-----------------|-----------------|
| SepFast Butyl-400 | 25 ml | 480101 |
| | 100 ml | 480102 |
| | 1 litre | 480103 |
| Disposable SepFast Butyl-400 column | 5 x 1 ml | 480104 |
| | 1 x 5 ml | 480105 |
| | 1 x 10 ml | 480106 |
| | 1 x 20 ml | 480107 |
| SepFast Butyl-500 | 25 ml | 480201 |
| | 100 ml | 480202 |
| | 1 litre | 480203 |
| Disposable SepFast Butyl-500 column | 5 x 1 ml | 480204 |
| | 1 x 5 ml | 480205 |
| | 1 x 10 ml | 480206 |
| | 1 x 20 ml | 480207 |
| SepFast Butyl-600 | 25 ml | 480301 |
| | 100 ml | 480302 |
| | 1 litre | 480303 |
| Disposable SepFast Butyl-600 column | 5 x 1 ml | 480304 |
| | 1 x 5 ml | 480305 |
| | 1 x 10 ml | 480306 |
| | 1 x 20 ml | 480307 |
| SepFast Pentyl-400 | 25 ml | 480401 |
| | 100 ml | 480402 |
| | 1 litre | 480403 |
| Disposable SepFast Pentyl-400 column | 5 x 1 ml | 480404 |
| | 1 x 5 ml | 480405 |
| | 1 x 10 ml | 480406 |
| | 1 x 20 ml | 480407 |
| SepFast Pentyl-500 | 25 ml | 480501 |
| | 100 ml | 480502 |
| | 1 litre | 480503 |
| Disposable SepFast Pentyl-500 column | 5 x 1 ml | 480504 |
| | 1 x 5 ml | 480505 |
| | 1 x 10 ml | 480506 |

| | | |
|--------------------------------------|-----------|--------|
| | 1 x 20 ml | 480507 |
| SepFast Pentyl-600 | 25 ml | 480601 |
| | 100 ml | 480602 |
| | 1 litre | 480603 |
| Disposable SepFast Pentyl-600 column | 5 x 1 ml | 480604 |
| | 1 x 5 ml | 480605 |
| | 1 x 10 ml | 480606 |
| | 1 x 20 ml | 480607 |
| SepFast Hexyl-400 | 25 ml | 480701 |
| | 100 ml | 480702 |
| | 1 litre | 480703 |
| Disposable SepFast Hexyl-400 column | 5 x 1 ml | 480704 |
| | 1 x 5 ml | 480705 |
| | 1 x 10 ml | 480706 |
| | 1 x 20 ml | 480707 |
| SepFast Hexyl-500 | 25 ml | 480801 |
| | 100 ml | 480802 |
| | 1 litre | 480803 |
| Disposable SepFast Hexyl-500 column | 5 x 1 ml | 480804 |
| | 1 x 5 ml | 480805 |
| | 1 x 10 ml | 480806 |
| | 1 x 20 ml | 480807 |
| SepFast Hexyl-600 | 25 ml | 480901 |
| | 100 ml | 480902 |
| | 1 litre | 480903 |
| Disposable SepFast Hexyl-600 column | 5 x 1 ml | 480904 |
| | 1 x 5 ml | 480905 |
| | 1 x 10 ml | 480906 |
| | 1 x 20 ml | 480907 |
| SepFast Phenyl-400 | 25 ml | 481001 |
| | 100 ml | 481002 |
| | 1 litre | 481003 |
| Disposable SepFast Phenyl-400 column | 5 x 1 ml | 481004 |
| | 1 x 5 ml | 481005 |
| | 1 x 10 ml | 481006 |
| | 1 x 20 ml | 481007 |
| SepFast Phenyl-500 | 25 ml | 481101 |
| | 100 ml | 481102 |
| | 1 litre | 481103 |
| Disposable SepFast Phenyl-500 column | 5 x 1 ml | 481104 |
| | 1 x 5 ml | 481105 |
| | 1 x 10 ml | 481106 |
| | 1 x 20 ml | 481107 |
| SepFast Phenyl-600 | 25 ml | 481201 |

| | | |
|--------------------------------------|-----------|--------|
| | 100 ml | 481202 |
| | 1 litre | 481203 |
| Disposable SepFast Phenyl-600 column | 5 x 1 ml | 481204 |
| | 1 x 5 ml | 481205 |
| | 1 x 10 ml | 481206 |
| | 1 x 20 ml | 481207 |
| <hr/> | | |
| SepFast Heptyl-400 | 25 ml | 481301 |
| | 100 ml | 481302 |
| | 1 litre | 481303 |
| Disposable SepFast Heptyl-400 column | 5 x 1 ml | 481304 |
| | 1 x 5 ml | 481305 |
| | 1 x 10 ml | 481306 |
| | 1 x 20 ml | 481307 |
| <hr/> | | |
| SepFast Heptyl-500 | 25 ml | 481401 |
| | 100 ml | 481402 |
| | 1 litre | 481403 |
| Disposable SepFast Heptyl-500 column | 5 x 1 ml | 481404 |
| | 1 x 5 ml | 481405 |
| | 1 x 10 ml | 481406 |
| | 1 x 20 ml | 481407 |
| <hr/> | | |
| SepFast Heptyl-600 | 25 ml | 481501 |
| | 100 ml | 481502 |
| | 1 litre | 481503 |
| Disposable SepFast Heptyl-600 column | 5 x 1 ml | 481504 |
| | 1 x 5 ml | 481505 |
| | 1 x 10 ml | 481506 |
| | 1 x 20 ml | 481507 |
| <hr/> | | |
| SepFast Octyl-400 | 25 ml | 481601 |
| | 100 ml | 481602 |
| | 1 litre | 481603 |
| Disposable SepFast Octyl-400 column | 5 x 1 ml | 481604 |
| | 1 x 5 ml | 481605 |
| | 1 x 10 ml | 481606 |
| | 1 x 20 ml | 481607 |
| <hr/> | | |
| SepFast Octyl-500 | 25 ml | 481701 |
| | 100 ml | 481702 |
| | 1 litre | 481703 |
| Disposable SepFast Octyl-500 column | 5 x 1 ml | 481704 |
| | 1 x 5 ml | 481705 |
| | 1 x 10 ml | 481706 |
| | 1 x 20 ml | 481707 |
| <hr/> | | |
| SepFast Octyl-600 | 25 ml | 481801 |
| | 100 ml | 481802 |
| | 1 litre | 481803 |

| | | |
|---|-----------|--------|
| Disposable SepFast Octyl- 600 column | 5 x 1 ml | 481804 |
| | 1 x 5 ml | 481805 |
| | 1 x 10 ml | 481806 |
| | 1 x 20 ml | 481807 |



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