Data sheet

SepFast™ 4HF (4 High Flow)

SepFast™ 6HF (6 High Flow)

1. Introduction

SepFast 4HF and 6HF are highly cross-linked plain agarose beads often used for fractionation of large biological molecules based on their molecular weights. Gel Filtration is a proven technique, which is widely used for size-based molecular separation. The media is also often used as good base matrix to immobilise affinity ligands.

The feature and selection guide are listed as follows:

Characteristics of SepFast 4HF / 6HF:

Matrix	4HF: 4% agarose beads (highly cross-linked) 6HF: 6% agarose beads (highly cross-linked)
Particle size (μm)	50 – 150 μm
Molecules to separate	4HF: Up to 4 x 10^7 Dalton 6HF: Up to 5 x 10^6 Dalton
Operating flow velocity	Normally 50 to 300 cm/hour
pH stability	3-13 (short term) and 4-11 (long term)
Working temperature	+4^{O}C to +30^{O}C
Chemical stability	All commonly used buffers; 20% ethanol
Avoid	Oxidizing agents
Storage	20% ethanol

2. Column packing

The typical compression factor is around 15%, i.e. 115% of the target bed volume should be prepared. Before packing a column, the medium need be washed with at least 3 volumes of deionised water to remove the storage reagent. It can be done in a sintered filter funnel under vacuum. De-gassed deionised water is recommended as the packing liquid. The general guideline is shown below:

1. Suspend the washed medium to de-gassed water to make a 50% v/v slurry.
2. Set the column up. Purge the bottom plunger off air bubbles by filling it with water.
3. Fully re-slurry the medium. Pour it in against the column wall to avoid trapping air bubbles.
4. Top the column off with the packing liquid.
5. Carefully connect the top plunger or any top adaptor.
6. Start to run the pump at low flowrate (e.g. 1.5 – 2 ml/min for 16 mm i.d. column) until the bed is fully settled. Keep running for 30 mins. Then gradually increase the flowrate until a sustainable pressure is reached. If the pressure continues to creep up, the flowrate should be reduced.
7. Stop the pump. Seal the bottom side of the column. Push the top plunger down until it is 2 mm below the marked level.

8. Test the packed column using tracer in water. Typically, 1% acetone with injection volume of 0.2 – 1% of the column volume at 20 – 30 cm/hr is used.

Packing tips: If the peak asymmetry is too low (e.g. <0.7), that means the medium is over-compressed. The final packing pressure need be reduced. If the peak asymmetry is too high (e.g. >1.5), the final packing pressure need be increased. If the HETP isn't good enough, increasing the packing pressure and/or running the column for a longer period should help.

3. Method optimization

We recommend the use of a buffer with an ionic strength equivalent to 0.15 M NaCl (or greater) to avoid any undesirable ionic interactions between the target molecules and the medium. In general, the recommended flow velocity is 10–30 cm/hour for gel filtration use. The lower the flow velocity the better the resolution. The sample volume should be within the range 0.1–1.0% of the packed bed volume. For certain easy-to-separate proteins, higher loading volume can be used.

Before applying a sample, the column should be equilibrated with 2 column volumes of buffer until a stable baseline is reached. Re-equilibration between runs is normally not necessary.

In the case that denatured proteins or lipids are not eluted within one column volume, the cleaning-in- place procedures should ensure the removal of these substances.

4. 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 surface of the medium. 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 methods work as a general guidance.

Method 1 - 0.5 M NaOH solution at a linear flow velocity of roughly 10-20 cm/hour, at a reversed flow direction and with a contact time of 1-2 hours, can be used to clean impurities bound to the medium.

Method 2 - Apply two bed volumes of 0.1–0.5% detergent in a basic or acidic solution at a linear flow velocity of roughly 10-20 cm/hour, at a reversed flow direction. Residual detergent should be removed by washing the column with five bed volumes of concentrated organic solvent.

Method 3 - Apply a concentrated organic solvent such as two bed volumes of 70% ethanol or 30% isopropanol at a linear flow velocity of roughly 10-20 cm/hour, at a reversed flow direction. To avoid the formation of air bubbles, organic solvents should be applied in increasing concentration gradients.

For all methods, after the CIP step, the column should be equilibrated with at least 3 column volumes of buffer before the next run.

Sanitization

Sanitization using at least 1 bed volume of 0.5-1.0 M NaOH, at a flow velocity of 10-20 cm/hour is recommended. Following sanitization, the column should be re-equilibrated with 3-5 bed volumes of buffer.

5. Storage

The media should be stored in 20% ethanol (long term) or 10 mM NaOH (short term) to prevent microbial growth. Store the media at a temperature of +2°C to +30°C.

6. Ordering information

Product	Quantity	Code no.
SepFast 4HF	100 ml	*540703-100ML*
	500 ml	*540703-500ML*
	1 litre	*540703-1L*
	5 litre	*540703-5L*
SepFast 6HF	100 ml	*540803-100ML*
	500 ml	*540803-500ML*
	1 litre	*540803-1L*
	5 litre	*540803-5L*

*For larger pack sizes, please contact info@biotoolomics.com

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