Data sheet

Q SepFast™ 6HF Plus
DEAE SepFast™ 6HF Plus
SP SepFast™ 6HF Plus
CM SepFast™ 6HF Plus

1. Introduction

Q SepFast 6HF Plus, DEAE SepFast 6HF Plus, SP SepFast 6HF Plus and CM SepFast 6HF Plus is a group of strong anion exchange, weak anion exchange, strong cation exchange and weak cation exchange media with tentacle chains, respectively. The binding capacity and binding kinetics is higher than IEX SepFast 6HF. The above ion-exchange chromatography media is particularly suitable for general large-scale purification of biological molecules in which increased binding capacity is desired.

This group of ion-exchange media has a balanced design among ligand density, loading capacity and separation power of individual components for large-scale biomanufacturing applications.

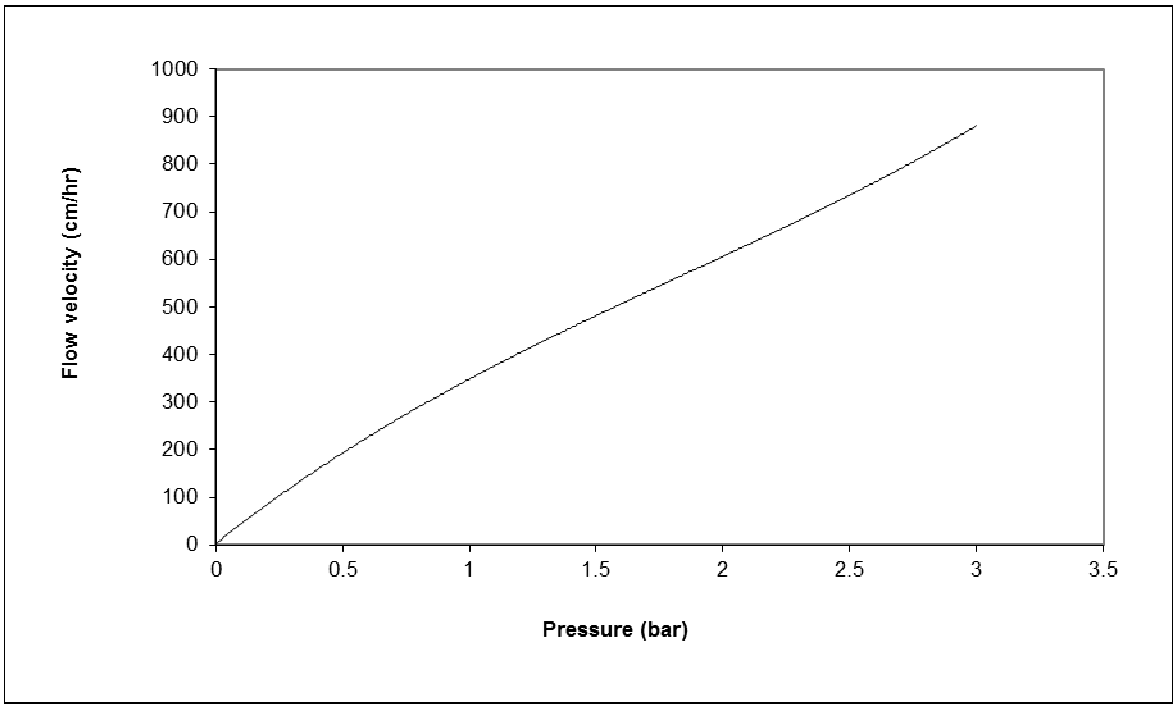
The base matrix is made of highly cross-linked 6% agarose and dextran with excellent flow property. It is very stable to most of the chemical conditions experienced in the bioprocessing industry.

2. Product properties

Characteristics of the ion-exchange SepFast 6HF Plus:

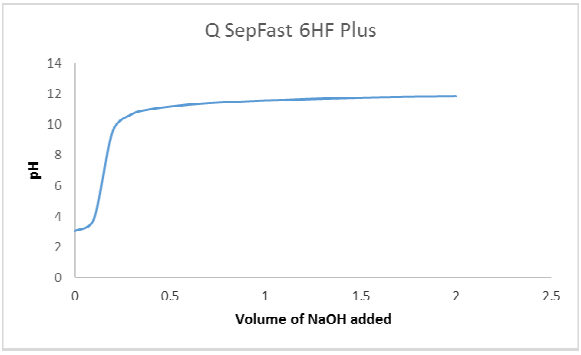
	Q SepFast 6HF Plus	DEAE SepFast 6HF Plus	SP SepFast 6HF Plus	CM SepFast 6HF Plus
Matrix	Highly cross-linked 6% agarose grafted with dextran chain			
Functional group	Quaternary ammonium strong anion	Diethylaminoethyl weak anion	Sulfopropyl strong cation	Carboxymethyl weak cation
Total ionic capacity	0.09-0.15 mmol/ml	0.08-0.13 mmol/ml	0.09-0.15 mmol/ml	0.08-0.13 mmol/ml
Dynamic binding capacity*	>130 mg BSA / ml	>130 mg BSA / ml	>130 mg lysozyme / ml	>130 mg lysozyme / ml
Particle size	50 - 150 μm			
Pressure-flow property**	>500 cm/hr at 3 bar			
Operational pressure	Up to 3 bar			
pH stability	2-14 (short term) and 3-12 (long term)		2-14 (short term) and 4-12 (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% isopropanol, 70% ethanol			
Avoid	Oxidizing agents, anionic detergents		Oxidizing agents, cationic detergents	
Storage	20% ethanol	20% ethanol	20% ethanol + 0.2 M sodium acetate	20% ethanol

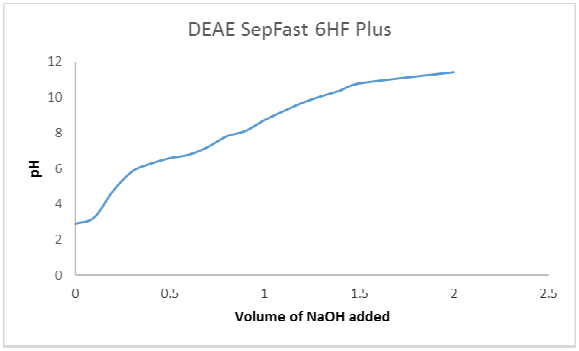
**Test condition: 10 mM buffer pH 7.5, 5 mg/ml protein to load, residence time 5 min, 50% breakthrough*
***Measured in a 32 mm ID column at a bed height of 15 cm using water at room temperature.*

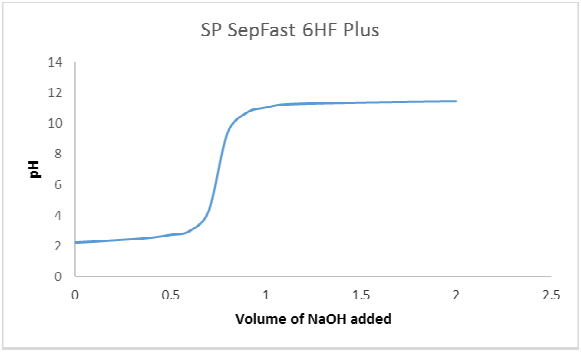


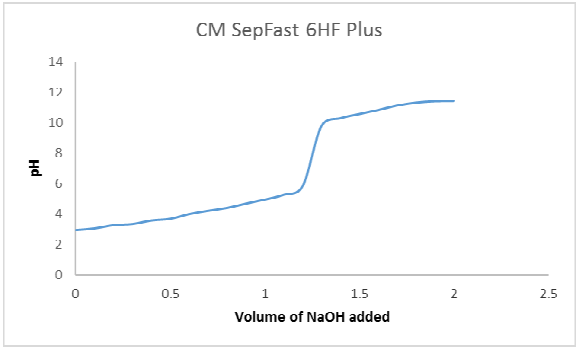
Pressure-flow property of the IEX media: The base matrix was packed to a 32 mm i.d. column with the final bed height of 15 cm. The mobile phase was deionised water. The test was conducted at room temperature.

Titration curves









3. Method optimization

We recommend scouting the parameters among loading capacity, flow velocity, binding pH, binding ionic strength, elution speed and gradient etc. Due to the fast pore accessibility of SepFast media, the binding step could be done in a faster flow velocity than that in the elution step. We recommend to pay special attention to optimize elution conditions to achieve the best separation power.

Strong ion exchange media maintain their charges (and thus their function) over a wide pH range whereas with weak ion exchange media the degree of dissociation and thus ion exchange capacity varies with pH. Therefore, it is more critical to optimize the pH if weak ion exchange media is used.

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.

4. Column packing

SepFast ion-exchange media is made of highly cross-linked beaded agarose with excellent mechanical strength. The media can be easily packed in any type of chromatography columns at any possible packing mode. The typical packing pressure is up to 3 bars.

5. Maintenance

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

Regeneration

After each run, elute any reversibly bound material either with a high ionic strength solution (e.g. 1M NaCl in buffer) or by increased pH.

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.

Salt with concentration up to 2 M can be used to clean the impurities bound by ionic interactions. 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.

6. Storage

The media should be stored in 20% ethanol (containing 0.2 M NaAC for strong cation exchange media) 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.

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