Data sheet

Q SepFast™ Large Beads **Q SepFast™ Large Beads Plus**

DEAE SepFast™ Large Beads **DEAE SepFast™ Large Beads Plus**

SP SepFast™ Large Beads **SP SepFast™ Large Beads Plus**

CM SepFast™ Large Beads **CM SepFast™ Large Beads Plus**

1. Introduction

Q (DEAE, SP, CM) SepFast Large Beads and Q (DEAE, SP, CM) SepFast Large Beads Plus are a group of ion exchange chromatography media with large particle sizes (100 – 350 μm). The base matrix shows excellent flow property and stable physical property.

The above ion-exchange chromatography media is particularly suitable for large-scale purification of biological molecules from viscous and/or crude culture brothes or for high speed flow-through polishing applications in which impure components are chromatographically adsorbed from the main product stream.

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. ***IEX SepFast Large Beads Plus offers increased binding capacity and faster binding kinetics than IEX SepFast Large Beads.***

The base matrix of SepFast Large Beads is made of highly cross-linked agarose with excellent flow property. The SepFast Large Beads Plus is made of highly cross-linked agarose grafted with dextran for increased binding capacity. They are very stable to most of the chemical conditions experienced in the bioprocessing industry.

2. Product properties

Characteristics of the IEX SepFast Large Beads:

	Q SepFast Large Beads	DEAE SepFast Large Beads	SP SepFast Large Beads	CM SepFast Large Beads
Matrix	Highly cross-linked agarose beads			
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
Particle size	100 - 350 μm			
Operational flow velocity*	>2000 cm/hr			
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

**Measured in a 32 mm ID column at a bed height of 15 cm using water at room temperature.*

Characteristics of the IEX SepFast Large Beads Plus:

	Q SepFast Large Beads Plus	DEAE SepFast Large Beads Plus	SP SepFast Large Beads Plus	CM SepFast Large Beads Plus
Matrix	Highly cross-linked agarose and dextran beads			
Functional group	Quaternary ammonium strong anion	Diethylaminoethyl weak anion	Sulfopropyl strong cation	Carboxymethyl weak cation
Total ionic capacity	0.1-0.2 mmol/ml	0.1-0.2 mmol/ml	0.1-0.2 mmol/ml	0.1-0.2 mmol/ml
Particle size	100 - 350 μm			
Operational flow velocity*	>2000 cm/hr			
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

**Measured in a 32 mm ID column at a bed height of 15 cm using water at room temperature.*

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 faster binding kinetics of SepFast Large Beads Plus, 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 Large Beads and SepFast Large Beads Plus 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 (flow pressure packing, axial compression packing or suction packing etc). 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. 2M 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^{o}C to +30^{o}C. Before use, equilibrate the media with at least 5 bed volumes of the running buffer.

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