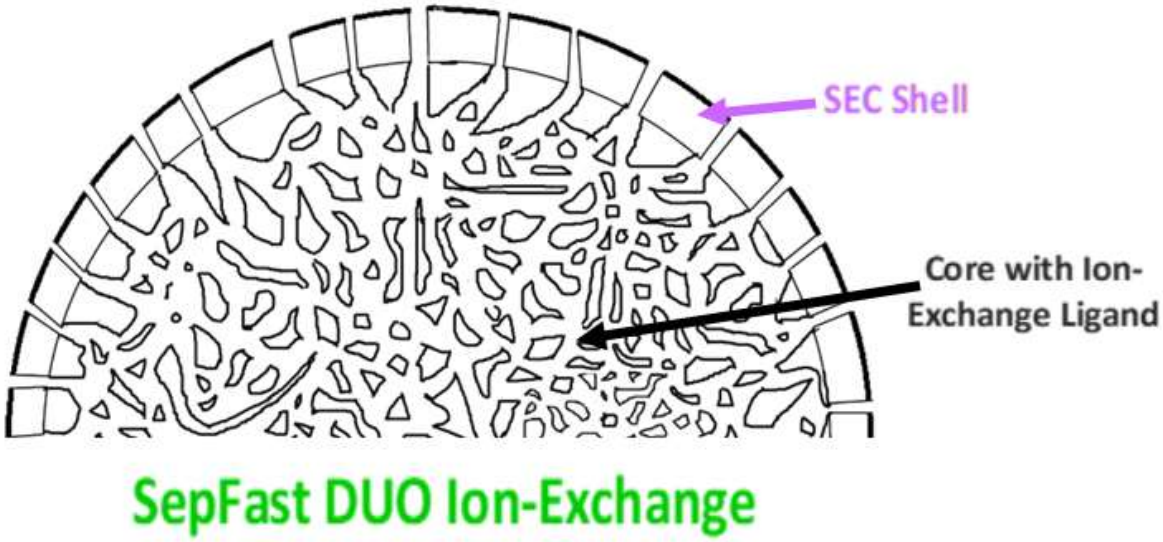
SepFast™ DUO Data sheet

SepFast™ DUO 150 Q	**SepFast™ DUO 150 S**
SepFast™ DUO 400 Q	**SepFast™ DUO 400 S**
SepFast™ DUO 700 Q	**SepFast™ DUO 700 S**
SepFast™ DUO 5000 Q	**SepFast™ DUO 5000 S**

SepFast DUO IEX is unique strong ion-exchange chromatography media utilising dual functionalities. Individual beads are coated with an inert polymer out-layer giving a size-exclusion effect. Inside the bead are strong anion-exchange or strong cation-exchange ligands. This type of novel media is designed for the selective purification of proteins or other molecules based on both molecular weight and charge. It is particularly effective and efficient for flow-through purification of antibodies, large proteins, viruses and viral-like-particles etc.



1. Properties

The SepFast DUO range of strong ion exchange chromatography media (with Q or S ligands) has a thin porous inert out-layer that excludes molecules with molecular weight >150 KDa (150 Q or S), >400 KDa (400 Q or S), >700 KDa (700 Q or S) and >5000 KDa (5000 Q or S). Molecules smaller than the designated size-exclusion level can penetrate the outer layer and are adsorbed, based on their charges, inside the bead.

The base matrix is a composite of polysaccharides that have been highly cross-linked. The media is stable in most of the chemical conditions experienced in the bioprocessing industry.

Table 1: Characteristics of SepFast DUO Ion-exchange Media:

Matrix	Beads of cross-linked polysaccharide composite
Functional group	Strong anion Q, $-N^{+}(CH_3)_3$ inside the bead of SepFast DUO Q Strong cation S, $-SO^{-}_3$ inside the bead of SepFast DUO S
Size-exclusion effect	SepFast DUO 150: blocking molecules larger than ~150 KDa SepFast DUO 400: blocking molecules larger than ~400 to 500 KDa SepFast DUO 700: blocking molecules larger than ~700 to 800 KDa SepFast DUO 5000: blocking molecules larger than ~5000 KDa
Small ionic capacity	0.07 – 0.15 mmol/ml medium
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
Avoid	Oxidizing agents, ionic detergents

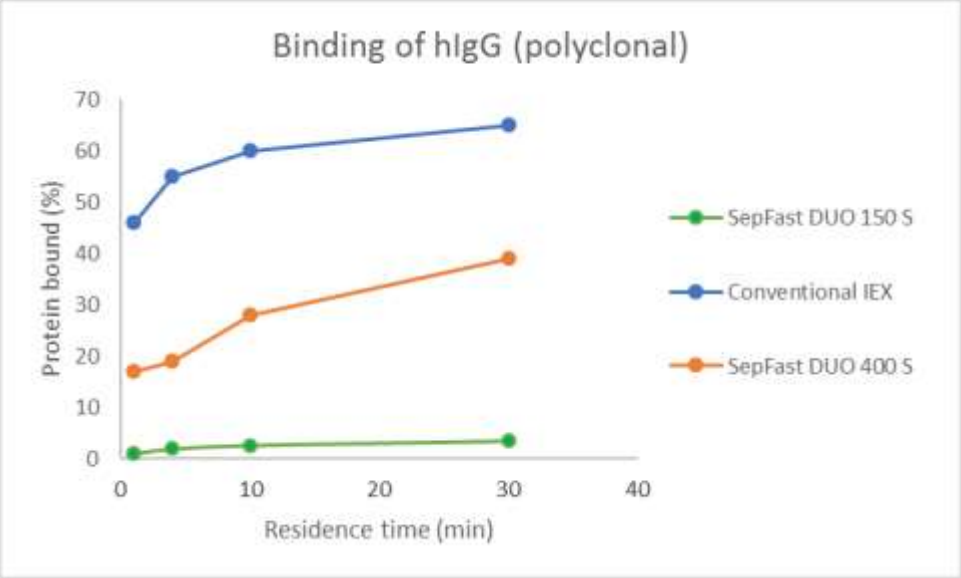


Figure 2: Comparison of the binding performance of conventional porous ion-exchange medium to SepFast DUO media having different size-exclusion shells. Note the very efficient exclusion of human immunoglobulin molecule (around 150 KDa) from the active binding core of SepFast 150 S.

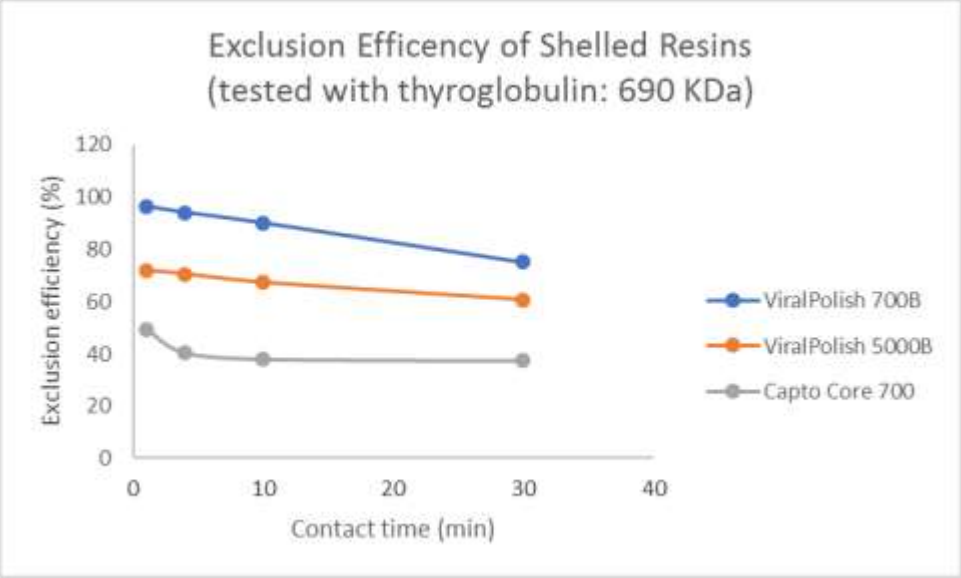


Figure 3: Size-exclusion effectiveness of SepFast DUO type of media is further demonstrated using shelled media having mixed-mode ligands inside. ViralPolish range of media has the same bead structure as SepFast DUO but the ligand inside is mixed-mode ligand tolerating high salt, designed to bind all smaller size impurities to provide a flow-through 'clean up' step.

2. Applications

SepFast DUO range of strong ion-exchange media can be used in polishing steps to remove smaller molecular weight impurities from antibodies or viruses etc.

The media can also be used for the purification of proteins in bind-elute mode if high molecular weight impurities need be removed from a target protein. In this case, the target molecule is bound inside but higher molecular weight impurities will straight flow to waste.

3. Operation

The loose media is stored in 20% ethanol (plus 0.2 M sodium acetate for strong cation exchange media) on delivery. It can be easily packed to any commercially available chromatography columns.

Column packing can be done in deionised water or low salt buffers using all the common methods. For flow packing, particular attention should be given to the maximum packing pressure. The typical packing pressure is 0.2 – 0.3 MPa (2-3 bar). Increase or decrease the packing pressure if the peak asymmetry becomes >1.5 or <0.7. Operate the column at a pressure lower than the maximum packing pressure.

Packing Efficiency Assessment

To check the quality of the packing and to monitor this during the working life of the column, column efficiency should be tested directly after packing, prior to re-use and if there is an observed deterioration in separation performance. The efficiency of a packed column is expressed in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (As). These values are easily determined by applying a sample such as 1% acetone solution to the column and using water as eluent. Sodium chloride can also be used as a test substance. Use a concentration of 2 M NaCl in water with 0.4 M NaCl in water as eluent. It is important that conditions and equipment are kept constant so that results are comparable. Changes in solute, solvent, eluent, sample volume, flow rate, liquid pathway, temperature, etc., will influence the results. A sample volume of less than 2.5% of the column volume and the flow velocity between 15 and 30 cm/h will give the optimal results.

4. Method optimization

We recommend scouting for optimal binding pH, ionic strength and flow velocity (i.e. residence time). We recommend special attention be paid to optimising the flow velocity to balance product yield and product purity.

In general, balancing product recovery against process throughput is the major consideration when optimizing a method. However, for the purification of shear-force sensitive molecules, the operational flow velocity needs to be optimised to minimise possible damage to the target molecule.

Tips:

- If unexpectedly high loss of product is noticed, consider using increased flow rates and/or increased ionic strength, or to adjust the pH to lower the charge of the target product.
- If too high level of impurities remain in flow-through mode, another medium with higher size-exclusion level may be tested at increased flow velocity. For example, some antibody systems may work better using SepFast DUO 400 instead of SepFast DUO 150.

- For AAV viruses, the first choice is SepFast DUO 700. For larger viruses, SepFast DUO 5000 is the first choice.

5. Process scale-up

The SepFast DUO range of media is designed for bioprocessing use with full regulatory support documents. Please contact us for further information.

6. Maintenance

Depending on individual applications, please see the following recommendations.

Note: when sodium hydroxide solution or organic solvent (e.g. 20% ethanol etc) is used, the flowrate must be less than 50% of the normal operational flowrate, because the column pressure will increase under these chemical conditions.

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 column 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 of concentration up to 2 M can be used to clean the impurities bound by ionic interactions. The contaminants bound hydrophobically can be removed by using 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.
Note: Long contact times should be avoided when using alcohols in acrylic columns.

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

7. Storage

The loose media or column should be stored in 20% ethanol to prevent microbial growth. Store the column at a temperature of +2°C to +8°C. After storage, equilibrate the column with at least 5 bed volumes of running buffer before use.

8. Ordering information

Product	Quantity	Code no.
SepFast DUO 150 Q	25 ml	510101-25ML
	100 ml	510101-100ML
	1 litre	510101-1L
Pre-packed column	5 x 1 ml	510101-5x1ML
	5 x 5 ml	510101-5x5ML
SepFast DUO 150 S	25 ml	510201-25ML
	100 ml	510201-100ML
	1 litre	510201-1L
Pre-packed column	5 x 1 ml	510201-5x1ML
	5 x 5 ml	510201-5x5ML
SepFast DUO 400 Q	25 ml	510102-25ML
	100 ml	510102-100ML
	1 litre	510102-1L
Pre-packed column	5 x 1 ml	510102-5x1ML
	5 x 5 ml	510102-5x5ML
SepFast DUO 400 S	25 ml	510202-25ML
	100 ml	510202-100ML
	1 litre	510202-1L
Pre-packed column	5 x 1 ml	510202-5x1ML
	5 x 5 ml	510202-5x5ML
SepFast DUO 700 Q	25 ml	510103-25ML
	100 ml	510103-100ML
	1 litre	510103-1L
Pre-packed column	5 x 1 ml	510103-5x1ML
	5 x 5 ml	510103-5x5ML
SepFast DUO 700 S	25 ml	510203-25ML
	100 ml	510203-100ML
	1 litre	510203-1L
Pre-packed column	5 x 1 ml	510203-5x1ML
	5 x 5 ml	510203-5x5ML
SepFast DUO 5000 Q	25 ml	510104-25ML
	100 ml	510104-100ML
	1 litre	510104-1L
Pre-packed column	5 x 1 ml	510104-5x1ML
	5 x 5 ml	510104-5x5ML
SepFast DUO 5000 S	25 ml	510204-25ML
	100 ml	510204-100ML

	1 litre	510204-1L
Pre-packed column	5 x 1 ml	510204-5x1ML
	5 x 5 ml	510204-5x5ML
Selection kit SepFast DUO Q	1 ml column each of SepFast DUO 150 Q, 400 Q, 700 Q and 5000 Q, respectively	510100-4x1ML
Selection kit SepFast DUO S	1 ml column each of SepFast DUO 150 S, 400 S, 700 S and 5000 S, respectively	510200-4x1ML

- Other column sizes (7 mm, 11 mm, 16 mm, 26 mm i.d. at various bed heights) available on request

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