SepFast™ DUO data sheet

SepFast™ DUO 150 Q, DEA, S, CM

SepFast™ DUO 5000 Q, DEA, S, CM

SepFast™ DUO 6000 Q, S

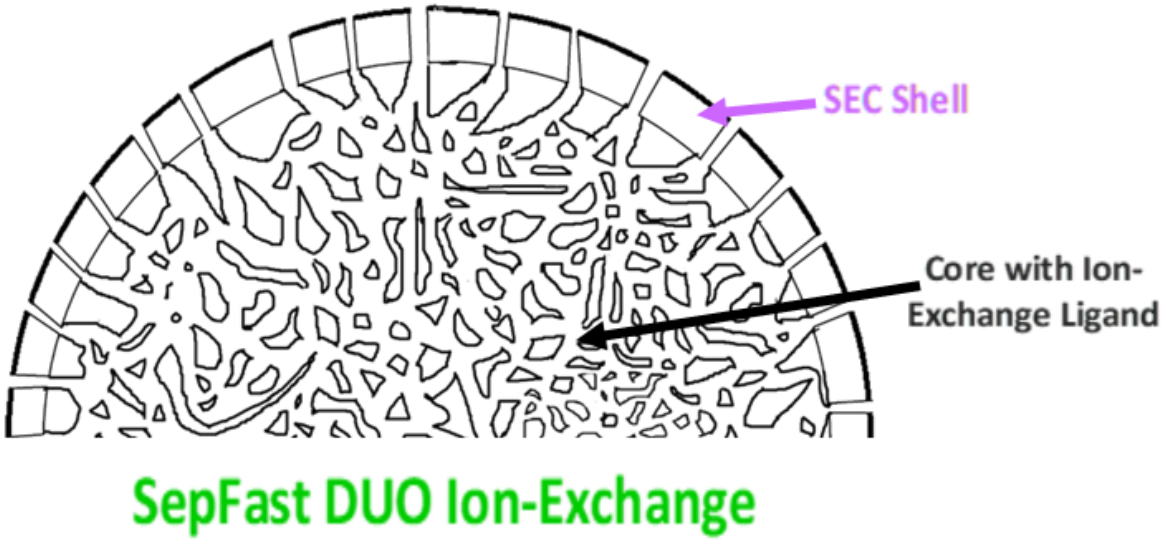
SepFast™ DUO 7000 Q, S

SepFast™ DUO 8000 Q, S

SepFast™ DUO 9000 Q, S

SepFast™ DUO 10000 Q, S

SepFast DUO IEX is unique 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 anion-exchange or cation-exchange ligands. This type of novel media is designed for the selective purification of proteins, viruses, DNAs, mRNAs etc based on both molecular weights and charges.



SepFast DUO Ion-Exchange

Large molecules, such as viruses, are excluded from the beads. The beads can be packed in a column where viruses will pass through the column bed and collected in flow through fraction, whilst impurities will be captured within the beads. This affords a very gentle purification process resulting in efficient clean up and high recovery of active virus particles.

SepFast DUO is available in a range of outer shells (i.e. different pore sizes) to best suit various purification needs based on the size of a product and the size of surrounding impurities.

1. Properties

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 bioprocessing industry. SepFast DUO can be used at process scale. Regulatory support files are available.

Selection Guide

SepFast DUO	**Application Guide**
SepFast DUO 150 Q (DEA, S, CM)	Good for removing low molecular weight impurities from monoclonal antibodies Q: strong anion-exchange; DEA: weak anion-exchange; S: strong cation-exchange; CM: weak cation-exchange
SepFast DUO 5000 Q (DEA, S, CM)	Good for small viruses such as small size AAVs, or for removing small DNA and RNA fragments etc. The pore size could block > 400KDa protein molecules if loading samples at high flow velocity (e.g. 1 mins contact time). A few million Dalton molecules may get through the shell if loading samples at very slow flow velocity (e.g. 10-30 mins contact time).
SepFast DUO 6000 Q (S) SepFast DUO 7000 Q (S)	Pore size larger than 5000 range. Good for larger AAVs, medium to large size viruses and VLPs, exosomes, DNA / RNA clearance (<1kb) etc.
SepFast DUO 8000 Q (S) SepFast DUO 9000 Q (S) SepFast DUO 10000 Q (S)	Good for even larger size viruses and VLPs, exosomes, DNA (>1kb) clearance etc, where impure species are very big and 7000 range couldn't remove.

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 Weak anion DEA, -diethylamine inside the bead of SepFast DUO DEA Strong cation S, $-SO^{-}_3$ inside the bead of SepFast DUO S Weak cation CM, -carboxylmethyl inside the bead of SepFast DUO CM
Particle size	50 – 150 µm (150 to 7000 range); 50 – 200 µm (8000 to 10000 range)
Operational pressure	Up to 3 bar (150 to 7000 range); up to 2 bar (8000 to 10000 range)
Ligand density	> 50 µmol / ml resin (for 5000 to 7000 range)
Protein binding capacity	Depends on the type of proteins and binding conditions; could be > 40 mg / ml resin for 5000 to 7000 range
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

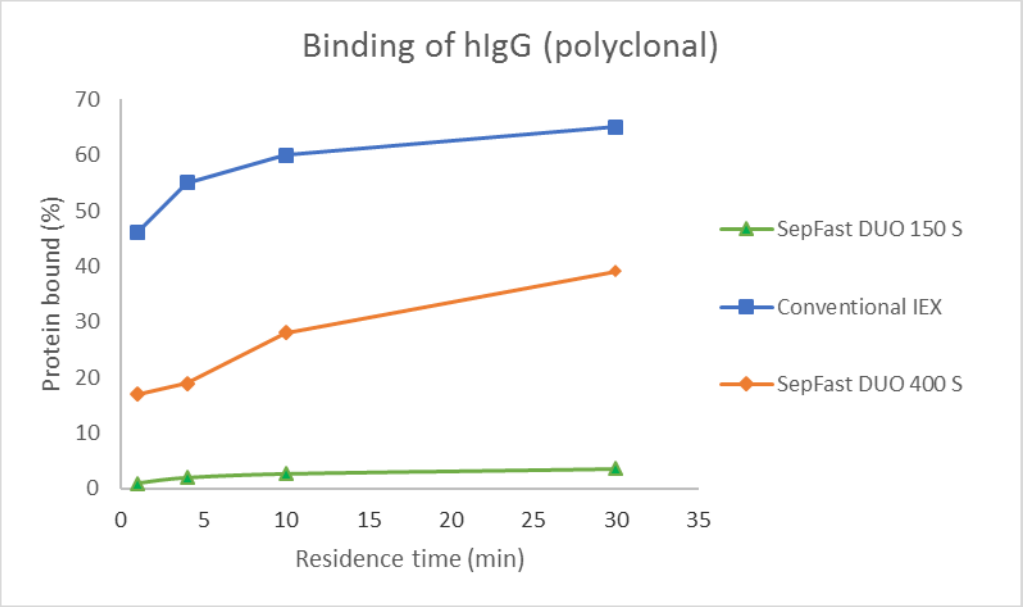


Figure: Comparison of the binding performance of conventional porous ion-exchange medium to SepFast DUO media having different size-exclusion shells. Note the very efficient exclusion of human immunoglobulin molecule (around 150 KDa) from the active binding core of SepFast 150 S.

2. Applications

SepFast DUO range of ion-exchange media can be used in flow-through mode to remove impurities smaller than a given product based on size and charge.

The media can also be used for the purification of molecules in bind-elute mode if impurities are larger than the target molecule. In this case, the target molecule is bound inside (to recover later) and the larger 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 0.1 – 0.3 MPa (1-3 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 1 M NaCl in water with 0.15 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 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. Residence time has a high impact to the efficiency of molecules' cut-off.

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 remains in flow-through fractions, another medium with higher size-exclusion level may be tested at increased flow velocity. For example, SepFast DUO 7000 is tested instead of SepFast DUO 6000.

- For AAV viruses, it's better to screen both SepFast DUO 5000 and 6000 range. For larger viruses, SepFast DUO 7000 range 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 loose media at ambient temperature. Store columns at a temperature of +2°C to +8°C. After storage, equilibrate each column with at least 5 bed volumes of running buffer before use.

8. Ordering information

Product	Quantity	Code no.
SepFast DUO 150 Q	10 ml	510101-10ML
	25 ml	510101-25ML
	100 ml	510101-100ML
	1 litre	510101-1L
	5 litre	510101-5L
	10 litre	510101-10L
Pre-packed column	5 x 1 ml	510101-5x1ML
	5 x 5 ml	510101-5x5ML
	7 mm x 100 mm	510101-7x100
	11 mm x 100 mm	510101-11x100
	16 mm x 100 mm	510101-16x100
	26 mm x 100 mm	510101-26x100
SepFast DUO 150 DEA	10 ml	510301-10ML
	25 ml	510301-25ML
	100 ml	510301-100ML
	1 litre	510301-1L
	5 litre	510301-5L
	10 litre	510301-10L
Pre-packed column	5 x 1 ml	510301-5x1ML
	5 x 5 ml	510301-5x5ML
	7 mm x 100 mm	510301-7x100
	11 mm x 100 mm	510301-11x100
	16 mm x 100 mm	510301-16x100
	26 mm x 100 mm	510301-26x100
SepFast DUO 150 S	10 ml	510201-10ML
	25 ml	510201-25ML
	100 ml	510201-100ML
	1 litre	510201-1L

	5 litre	510201-5L
	10 litre	510201-10L
Pre-packed column	5 x 1 ml	510201-5x1ML
	5 x 5 ml	510201-5x5ML
	7 mm x 100 mm	510201-7x100
	11 mm x 100 mm	510201-11x100
	16 mm x 100 mm	510201-16x100
	26 mm x 100 mm	510201-26x100
SepFast DUO 150 CM	10 ml	510401-10mL
	25 ml	510401-25ML
	100 ml	510401-100ML
	1 litre	510401-1L
	5 litre	510401-5L
	10 litre	510401-10L
Pre-packed column	5 x 1 ml	510401-5x1ML
	5 x 5 ml	510401-5x5ML
	7 mm x 100 mm	510401-7x100
	11 mm x 100 mm	510401-11x100
	16 mm x 100 mm	510401-16x100
	26 mm x 100 mm	510401-26x100
SepFast DUO 5000 Q	10 ml	510104-10ML
	25 ml	510104-25ML
	100 ml	510104-100ML
	1 litre	510104-1L
	5 litre	510104-5L
	10 litre	510104-10L
Pre-packed column	5 x 1 ml	510104-5x1ML
	5 x 5 ml	510104-5x5ML
	7 mm x 100 mm	510104-7x100
	11 mm x 100 mm	510104-11x100
	16 mm x 100 mm	510104-16x100
	26 mm x 100 mm	510104-26x100
SepFast DUO 5000 DEA	10 ml	510304-10ML
	25 ml	510304-25ML
	100 ml	510304-100ML
	1 litre	510304-1L
	5 litre	510304-5L

	10 litre	510304-10L
Pre-packed column	5 x 1 ml	510304-5x1ML
	5 x 5 ml	510304-5x5ML
	7 mm x 100 mm	510304-7x100
	11 mm x 100 mm	510304-11x100
	16 mm x 100 mm	510304-16x100
	26 mm x 100 mm	510304-26x100
SepFast DUO 5000 S	10 ml	510204-10ML
	25 ml	510204-25ML
	100 ml	510204-100ML
	1 litre	510204-1L
	5 litre	510204-5L
	10 litre	510204-10L
Pre-packed column	5 x 1 ml	510204-5x1ML
	5 x 5 ml	510204-5x5ML
	7 mm x 100 mm	510204-7x100
	11 mm x 100 mm	510204-11x100
	16 mm x 100 mm	510204-16x100
	26 mm x 100 mm	510204-26x100
SepFast DUO 5000 CM	10 ml	510404-10ML
	25 ml	510404-25ML
	100 ml	510404-100ML
	1 litre	510404-1L
	5 litre	510404-5L
	10 litre	510404-10L
Pre-packed column	5 x 1 ml	510404-5x1ML
	5 x 5 ml	510404-5x5ML
	7 mm x 100 mm	510404-7x100
	11 mm x 100 mm	510404-11x100
	16 mm x 100 mm	510404-16x100
	26 mm x 100 mm	510404-26x100
SepFast DUO 6000 Q	25 ml	510105-25ML
	100 ml	510105-100ML
	1 litre	510105-1L
	5 litre	510105-5L
	10 litre	510105-10L
Pre-packed column	5 x 1 ml	510105-5x1ML

	5 x 5 ml	510105-5x5ML
	7 mm x 100 mm	510105-7x100
	11 mm x 100 mm	510105-11x100
	16 mm x 100 mm	510105-16x100
	26 mm x 100 mm	510105-26x100
SepFast DUO 6000 S	25 ml	510205-25ML
	100 ml	510205-100ML
	1 litre	510205-1L
	5 litre	510205-5L
	10 litre	510205-10L
Pre-packed column	5 x 1 ml	510205-5x1ML
	5 x 5 ml	510205-5x5ML
	7 mm x 100 mm	510205-7x100
	11 mm x 100 mm	510205-11x100
	16 mm x 100 mm	510205-16x100
	26 mm x 100 mm	510205-26x100
SepFast DUO 7000 Q	25 ml	510106-25ML
	100 ml	510106-100ML
	1 litre	510106-1L
	5 litre	510106-5L
	10 litre	510106-10L
Pre-packed column	5 x 1 ml	510106-5x1ML
	5 x 5 ml	510106-5x5ML
	7 mm x 100 mm	510106-7x100
	11 mm x 100 mm	510106-11x100
	16 mm x 100 mm	510106-16x100
	26 mm x 100 mm	510106-26x100
SepFast DUO 7000 S	25 ml	510206-25ML
	100 ml	510206-100ML
	1 litre	510206-1L
	5 litre	510206-5L
	10 litre	510206-10L
Pre-packed column	5 x 1 ml	510206-5x1ML
	5 x 5 ml	510206-5x5ML
	7 mm x 100 mm	510206-7x100
	11 mm x 100 mm	510206-11x100
	16 mm x 100 mm	510206-16x100

	26 mm x 100 mm	510206-26x100
SepFast DUO 8000 Q	25 ml	510107-25ML
	100 ml	510107-100ML
	1 litre	510107-1L
	5 litre	510107-5L
	10 litre	510107-10L
Pre-packed column	5 x 1 ml	510107-5x1ML
	5 x 5 ml	510107-5x5ML
	7 mm x 100 mm	510107-7x100
	11 mm x 100 mm	510107-11x100
	16 mm x 100 mm	510107-16x100
	26 mm x 100 mm	510107-26x100
SepFast DUO 8000 S	25 ml	510207-25ML
	100 ml	510207-100ML
	1 litre	510207-1L
	5 litre	510207-5L
	10 litre	510207-10L
Pre-packed column	5 x 1 ml	510207-5x1ML
	5 x 5 ml	510207-5x5ML
	7 mm x 100 mm	510207-7x100
	11 mm x 100 mm	510207-11x100
	16 mm x 100 mm	510207-16x100
	26 mm x 100 mm	510207-26x100
SepFast DUO 9000 Q	25 ml	510108-25ML
	100 ml	510108-100ML
	1 litre	510108-1L
	5 litre	510108-5L
	10 litre	510108-10L
Pre-packed column	5 x 1 ml	510108-5x1ML
	5 x 5 ml	510108-5x5ML
	7 mm x 100 mm	510108-7x100
	11 mm x 100 mm	510108-11x100
	16 mm x 100 mm	510108-16x100
	26 mm x 100 mm	510108-26x100
SepFast DUO 9000 S	25 ml	510208-25ML
	100 ml	510208-100ML
	1 litre	510208-1L

	5 litre	510208-5L
	10 litre	510208-10L
Pre-packed column	5 x 1 ml	510208-5x1ML
	5 x 5 ml	510208-5x5ML
	7 mm x 100 mm	510208-7x100
	11 mm x 100 mm	510208-11x100
	16 mm x 100 mm	510208-16x100
	26 mm x 100 mm	510208-26x100
SepFast DUO 10000 Q	25 ml	510109-25ML
	100 ml	510109-100ML
	1 litre	510109-1L
	5 litre	510109-5L
	10 litre	510109-10L
Pre-packed column	5 x 1 ml	510109-5x1ML
	5 x 5 ml	510109-5x5ML
	7 mm x 100 mm	510109-7x100
	11 mm x 100 mm	510109-11x100
	16 mm x 100 mm	510109-16x100
	26 mm x 100 mm	510109-26x100
SepFast DUO 10000 S	25 ml	510209-25ML
	100 ml	510209-100ML
	1 litre	510209-1L
	5 litre	510209-5L
	10 litre	510209-10L
Pre-packed column	5 x 1 ml	510209-5x1ML
	5 x 5 ml	510209-5x5ML
	7 mm x 100 mm	510209-7x100
	11 mm x 100 mm	510209-11x100
	16 mm x 100 mm	510209-16x100
	26 mm x 100 mm	510209-26x100

- Other column sizes (50-450 mm column diameter at various bed heights) available on request

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