Data sheet

MabPolish® DUO

(150A, 150C)

(400A, 400C)

MabPolish DUO is a novel range of mixed mode chromatography media coated with inert size-exclusion out-layers. The media is designed for selective removal of host cell proteins or other molecules with molecular weights <150 KDa. It is particularly suitable for flow-through purification of monoclonal antibodies or other proteins larger than 150 KDa.

1. Properties

MabPolish DUO is a class of special mixed-mode chromatography media having inert out-layers that prevent larger molecules from getting in, i.e. the media has dual functionality with the benefit of increased selectivity. Inside the beads, mixed-mode ligands are immobilised to give strong binding to a broad range of proteins at wide pH range.

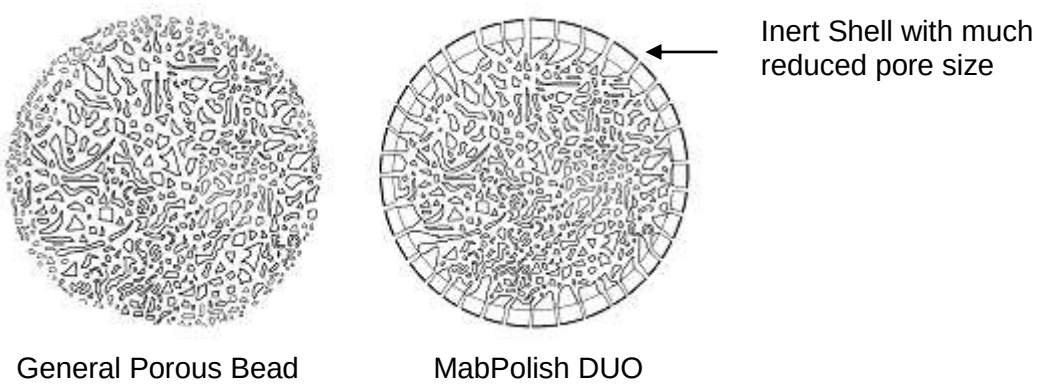

Figure 1: Illustration of the bead structure of MabPolish DUO material

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

Table 1: Characteristics of MabPolish DUO:

Matrix	Beads of cross-linked polysaccharide composite
Particle size	50 - 200 μm
Functional group	150A and 400A: anion mixed mode ligand 150C and 400C: cation mixed mode ligand
Size exclusion effect of the out-layer	150A and 150C: excluding 150 KDa molecules 400A and 400C: excluding 400 KDa molecules
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, detergents

2. Applications

MabPolish DUO can be used in flow-through mode to remove impurities of less than 150 KDa from monoclonal antibodies or other antibody materials. Examples of the possible application scenario could be:

- After an antibody is first purified by affinity chromatography media (e.g. Protein A, Protein G or Protein L etc);
- After an antibody is first purified by ion-exchangers;
- After an antibody is first processed through precipitation;
- Direct removal of impurities from crude antibody materials
- After an antibody is modified (e.g. conjugated etc)

The typical working condition:

- Common working pH range such as pH 4 to 8 can be used, though an optimised pH could give the best performance;
- Typical buffers include tris/HCl, sodium acetate buffer, sodium phosphate buffer etc
- Typical ionic strength is 0.02 M – 0.5 M salt

Please see Section 4 for process optimisation guidance to produce the best performance to individual proteins.

3. Operations

The loose media is stored in 20% ethanol 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. 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 most 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.

The typical working pH is 6 to 8 for 150A and 400A, and is 3.5 to 5 for 150C and 400C. The typical salt concentration is 0.1 to 0.3 M.

MabPolish DUO 400A and 400C is normally deployed to remove impurities similar or larger than 150 KDa antibodies. It is recommended to run MabPolish DUO 400A and 400C at increased flow velocities (e.g. 1 to 2 minutes residence time) to minimise the product loss.

Tips: If unexpected high loss of product is noticed, consider to run at increased flow rates and/or decreased ionic strength, or to adjust the pH to lower the charge of the target product.

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

5. Process scaling up

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

6. Maintenance

Depending on the individual applications, please see the following recommendations.

Note: when sodium hydroxide solution or organic solvent (e.g. 20% ethanol or 30% IPA 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 with 0.5 M NaOH.

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.

Reverse the flow to introduce the following liquid from the bottom of the column.

- 1 M acetic acid (+ 30% isopropanol if necessary) followed by
- 1 M NaCl (or water) followed by
- 1 M NaOH followed by
- 2 M NaCl followed by
- Equilibration buffer

Longer contact time in the acetic acid step and NaOH step may improve the CIP performance.

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. Order information

Product	Quantity	Code no.
MabPolish DUO 150A	25 ml	270301-25ML
	100 ml	270301-100ML
	1 litre	270301-1L
Pre-packed column	5 x 1 ml	270301-5x1ML
	1 x 10 ml	270301-1x10ML
MabPolish DUO 150C	25 ml	270302-25ML
	100 ml	270302-100ML
	1 litre	270302-1L
Pre-packed column	5 x 1 ml	270302-5x1ML
	1 x 10 ml	270302-1x10ML
MabPolish DUO 400A	25 ml	270303-25ML
	100 ml	270303-100ML
	1 litre	270303-1L
Pre-packed column	5 x 1 ml	270303-5x1ML
	1 x 10 ml	270303-1x10ML
MabPolish DUO 400C	25 ml	270304-25ML
	100 ml	270304-100ML
	1 litre	270304-1L
Pre-packed column	5 x 1 ml	270304-5x1ML
	1 x 10 ml	270304-1x10ML
MabPolish Selection Kit	6 x 1 ml	270000-6x1ML
	(1 ml each of MabPolish Type I, Type II, MabPolish DUO 150A, 150C, 400A, 400C)	

Note: other volumes available on request

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