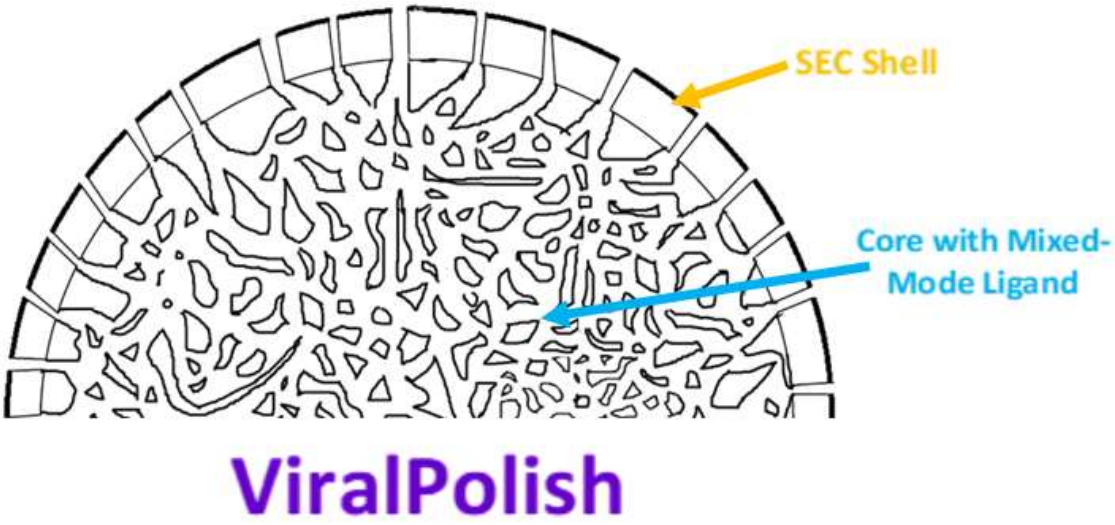
ViralPolish Data Sheet

ViralPolish®

BioToolomics developed ViralPolish® chromatography media specifically for virus purification; Comprising dual layer agarose beads with an inert external shell with tightly controlled pore size and internal polyfunctional ligands for rapid high capacity binding of impurities.



Large particles, such as viruses, are excluded from the beads. The beads can be packed in a column where the virus will pass through the column bed and collected in the 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.

ViralPolish® is available in three different outer shell pore sizes, and two different impurity binding chemistries (see table below).

1. Properties

ViralPolish range of chromatography media has a thin porous inert out-layer that excludes molecules with molecular weight >5000 KDa (5000A and 5000B), >700 KDa (700A and 700B), >400 KDa (400A and 400B), respectively. Molecules smaller than the designated size-exclusion level can penetrate the outer layer and are adsorbed by the mixed-mode ligands in the core.

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.

Selection Guide

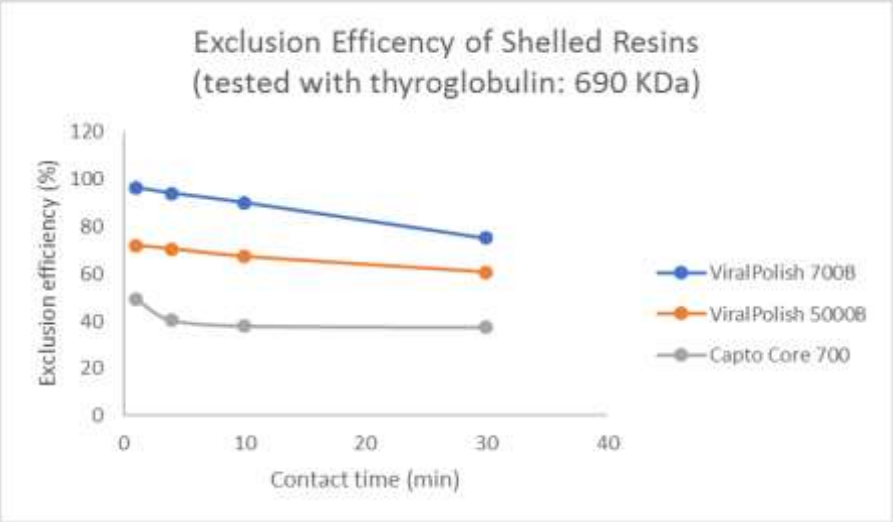
Shelled Mixed-Mode Resin	Application Guide	Key Features
ViralPolish™ 5000A ViralPolish™ 5000B	Good for lentivirus, adeno virus, VLPs and other viruses of large sizes AAV may be purified if very high molecular-weight impurity (e.g. truncated AAV or empty capsid) need be removed.	The inert out shell has molecular cut-off of 5000 KDa, 700 KDa and 400 KDa, respectively, to block viral particles of different sizes. "A": Mixed-mode anion ligand with mild hydrophobicity; can be easily re-generated and re-used; but its binding capacity may be compromised. "B": Mixed-mode anion ligand with strong hydrophobicity; have higher loading capacity but is difficult to re-generate. They are more suitable for single-use.
ViralPolish™ 700A ViralPolish™ 700B	Good for AAVs, other viruses and VLPs of similar sizes, plasmids etc	
ViralPolish™ 400A ViralPolish™ 400B	Good for certain vaccine antigens, large proteins etc	

Note: other mixed-mode ligands are available on request.

Product characteristics

Particle size	50 – 150 μm
Ligand	Mixed-mode
Ligand density	> 50 μmol / ml resin
Protein binding capacity	Depends on the type of proteins and binding conditions; could be > 40 mg / ml resin
Operational pressure	Up to 3 bar
Flow velocity	Up to 500 cm/hr
Chemical stability	Stable in most common aqueous buffers
pH stability	2-14 (<2 h) 3-12 (long term)
Storage	20% ethanol at 4°C

The pore size distribution of the outer layer is critical to prevent molecules of a given molecular weight from binding to exposed 'active core'. ViralPolish® has tightly controlled pores giving best in class exclusion of virus and large molecules, resulting in significantly higher recovery of active viral particles.



2. Applications

This media is ideal for rapid clean-up, either as a first step or as a second step, to purify virus particles. The beads can also be used in batch mode; when added to a flask of virus containing media the beads will selectively allow small molecules to enter and be retained, whilst the virus is excluded and remains in the external media.

A typical purification strategy for virus would be to use ViralPolish® as an initial step, to clean up the sample, prior to binding and elution, typically from an anion exchange media designed for virus purification such as SepFast Supor Q.

Alternatively, very dilute samples can be purified by first passing through a SepFast Supor Q anion exchange column to concentrate the sample followed by passing through ViralPolish column to remove residual impurities.

3. Operation

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 (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, ViralPolish 5000 may be used instead of viralPolish 700.

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

5. Process scale-up

ViralPolish 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.

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.

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. Order information

Product	Quantity	Code no.
ViralPolish 5000A	25 ml	270501-25ML
	100 ml	270501-100ML
	1 litre	270501-1L
Pre-packed column	5 x 1 ml	270501-5x1ML
	5 x 5 ml	270501-5x5ML
ViralPolish 5000B	25 ml	270502-25ML
	100 ml	270502-100ML
	1 litre	270502-1L
Pre-packed column	5 x 1 ml	270502-5x1ML
	5 x 5 ml	270502-5x5ML
ViralPolish 700A	25 ml	270601-25ML
	100 ml	270601-100ML
	1 litre	270601-1L
Pre-packed column	5 x 1 ml	270601-5x1ML
	5 x 5 ml	270601-5x5ML
ViralPolish 700B	25 ml	270602-25ML
	100 ml	270602-100ML
	1 litre	270602-1L
Pre-packed column	5 x 1 ml	270602-5x1ML
	5 x 5 ml	270602-5x5ML
ViralPolish 400A	25 ml	270401-25ML
	100 ml	270401-100ML

	1 litre	270401-1L
Pre-packed column	5 x 1 ml	270401-5x1ML
	5 x 5 ml	270401-5x5ML
ViralPolish 400B	25 ml	270402-25ML
	100 ml	270402-100ML
	1 litre	270402-1L
Pre-packed column	5 x 1 ml	270402-5x1ML
	5 x 5 ml	270402-5x5ML
ViralPolish Selection Kit	6 x 1 ml (1 ml each of ViralPolish 5000A, 5000B, 700A, 700B, 400A and 400B)	270001-6x1ML

Note: other column sizes available on request

BioToolomics Ltd
Unit 30A, Number 1 Industrial Estate
Consett
County Durham, DH8 6TJ
United Kingdom

www.biotoolomics.com