ProteoSEC 3-70KDa Preparative Resin

#Cat: NB-45-0218-5L Size: 5l
#Cat: NB-45-0218-1L Size: 1l
#Cat: NB-45-0218-10L Size: 10l
#Cat: NB-45-0218-25ml Size: 25ml
#Cat: NB-45-0218-150ml Size: 150ml

1. Introduction

ProteoSEC Preparative Resin is specially designed for high resolution and high recovery fractionation of biological molecules based on their molecular weights. Gel Filtration is a proven technique, which is widely used for size-based molecular separation.
ProteoSEC Preparative Resin has a balanced design to offer high recovery and high selectivity according to individual applications. The core advantages are:

- High resolution
- High selectivity
- High recovery
- Highly scalable
- Excellent physical and mechanical stability
- Cost effective

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

The feature and selection guide is listed as follows:

	ProteoSEC Preparative Resin	
	NB-45-0218-1L (ProteoSEC 3-70 Preparative Resin)	**NB-45-0219-1L (ProteoSEC 6-600 Preparative Resin)**
Separation range	$3x10^3$ - $7x10^4$	$6x10^3$ - $6x10^5$
Application	High selectivity, high resolution, high recovery, suitable for use in medium to higher pressuresystems	
Particle Size (µm)	20-50	20-50
Format supplied	Loose resin and pre-packed columns	

Characteristics of ProteoSEC Preparative Resin:

	NB-45-0218-1L NB-45-0219-1L
Matrix	Highly cross-linked polysaccharide composite of agarose and dextran
Particle size (µm)	Around 35 µm (in the range of 20 – 50 µm)
Operational pressure	Typically 3 to 4 bar (0.3 – 0.4 MPa, 42 – 56 psi)
Operating flow velocity	Normally 10-50 cm/hour
pH stability	2-14 (short term) and 3-12 (long term)
Working temperature	+4°C to +30°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
Storage	20% ethanol

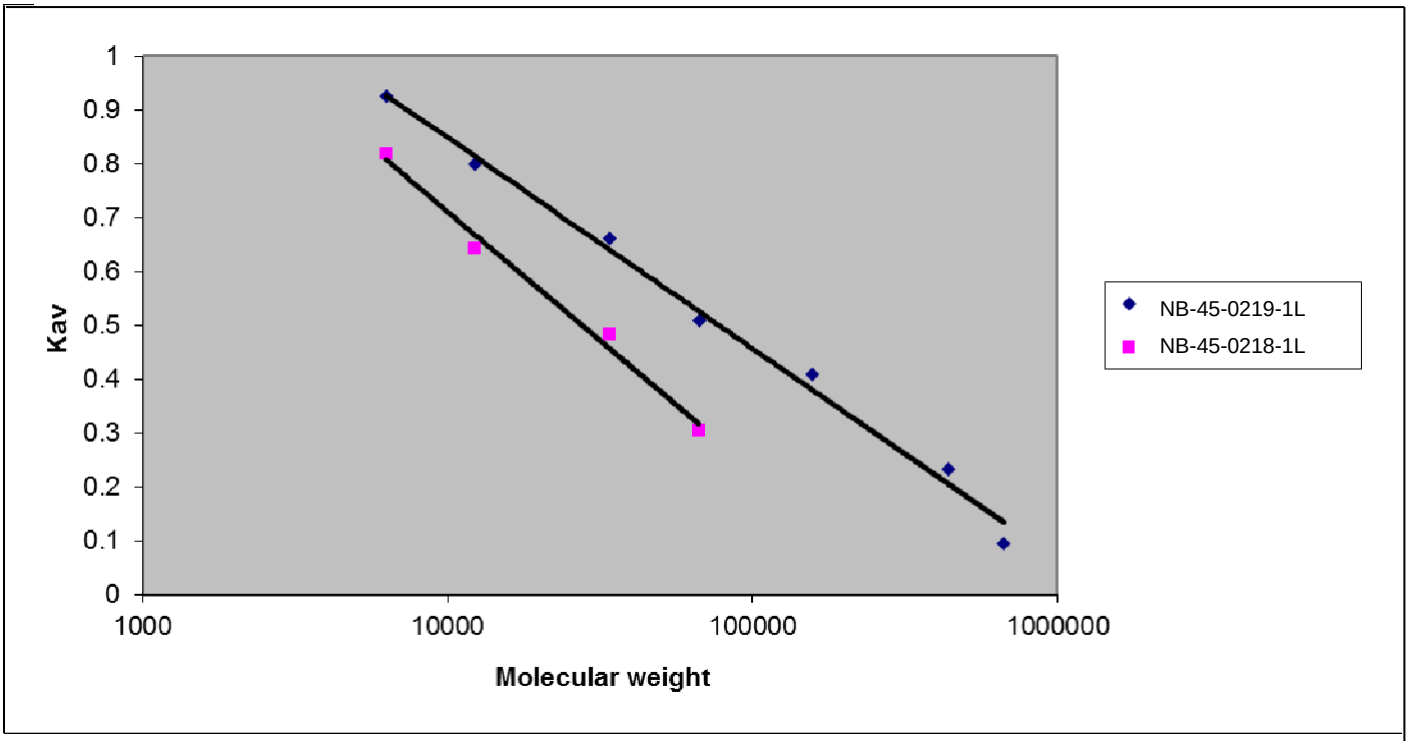
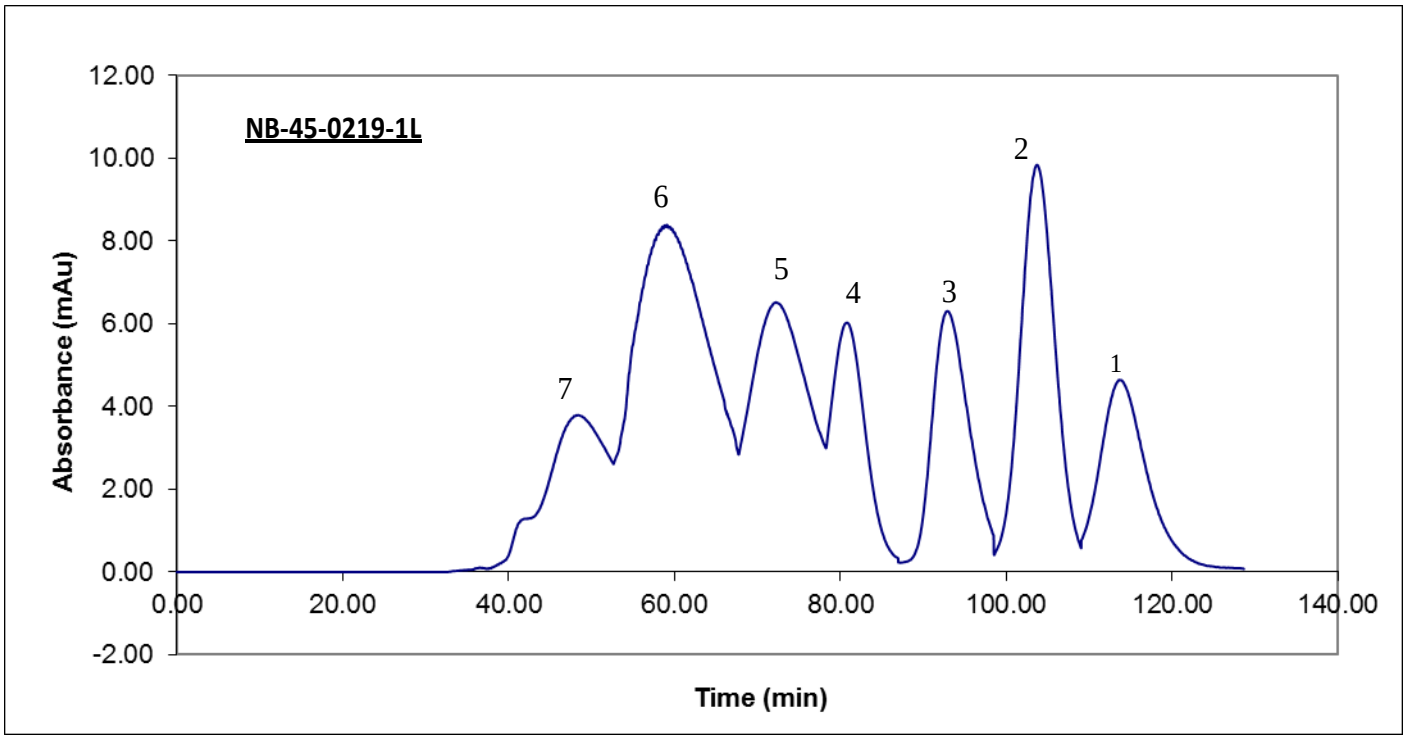


Figure 1: Selectivity curves of NB-45-0219-1L and NB-45-0218-1L. A broad range of molecules from 6,000 to 600,000 can be separated.



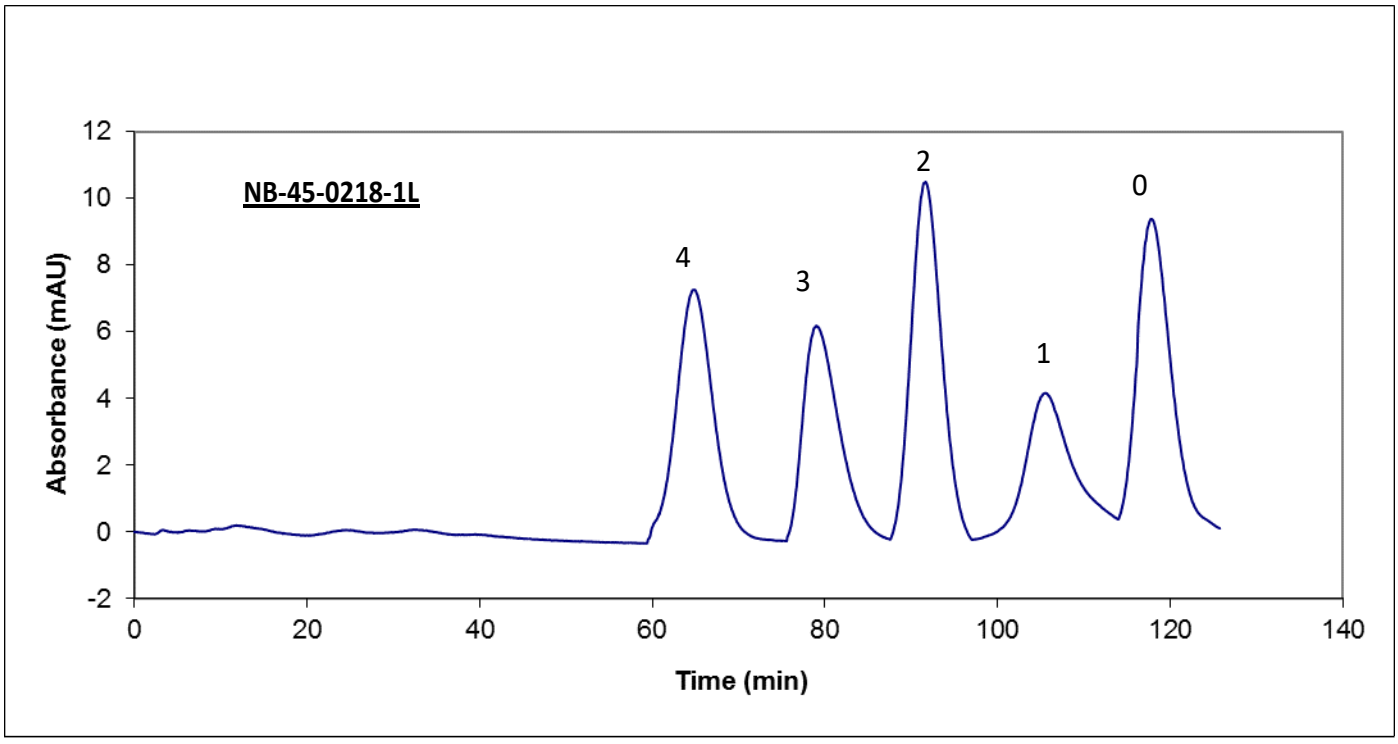


Figure 2: Separation of test substances on a 16/600 gel filtration column packed with NB-45-0219-1L and NB-45-0218-1L, respectively. Flowrate: 1 ml/min (30 cm/hr); sample loading 0.5 ml; mobile phase: PBS (phosphate buffered saline); model proteins 0: vitamin B-12 (1200); 1: aprotinin (M_r 6500); 2: cytochrome c (M_r 12300); 3: β-lactoglobulin (M_r 35000); 4: BSA (M_r 67000);5: ɣ-globulin IgG (M_r 158000; 6: apoferritin (M_r 440000); 7: thyroglobulin (M_r 669000)

2. Column packing

The loose medium is supplied in 20% ethanol. The typical compression factor is around 15%, i.e. 115% of the target bed volume should be prepared. Before packing a column, the medium need be washed with at least 3 volumes of deionised water to remove the ethanol. It can be done in a sintered filter funnel under vacuum. De-gassed deionised water is recommended as the packing liquid. The general guideline is shown below:

1. Suspend the washed medium to de-gassed water to make a 50% v/v slurry.
2. Set the column up. Purge the bottom plunger off air bubbles by filling it with water.
3. Fully re-slurry the medium. Pour it in against the column wall to avoid trapping air bubbles.
4. Top the column off with the packing liquid.
5. Carefully connect the top plunger or any top adaptor.
6. Start to run the pump at low flowrate (e.g. 1.5 – 2 ml/min for 16 mm i.d. column) until the bed is fully settled. Keep running for 30 mins. Then gradually increase the flowrate until a sustainable pressure is reached over 1 to 2 hr period. If the pressure continues to creep up, the flowrate should be reduced. The typical final pressure for NB-45-0219-1L is around 0.5 MPa at 60 cm bed height. The final pressure for NB-45-0218-1L could be 0.9 – 1 MPa at 60 cm bed height. Mark the level of the finally packed bed.
7. Stop the pump. Seal the bottom side of the column. Push the top plunger down until it is 2mm below the marked level.
8. Test the packed column using tracer in water. Typically, 1% acetone with injection volumeof 0.2 – 1% of the column volume at 30 – 60 cm/hr is used.

Packing tips: If the peak asymmetry is too low (e.g. <0.7), that means the medium is over-compressed. The final packing pressure need be reduced. If the peak asymmetry is too high (e.g. >1.5), the final packing pressure need be increased. If the HETP isn't good enough, increasing the packing pressure and/or running the column for a longer period should help.

3. Method optimization

We recommend the use of a buffer with an ionic strength equivalent to 0.15 M NaCl (or greater) to avoid any undesirable ionic interactions between the target molecules and the medium. In general, the recommended flow velocity is 10–50 cm/hour. The lower the flow velocity the better the resolution. The sample volume should be within the range 0.1–1.0% of the packed bed volume.
For certain easy-to-separate proteins, higher loading volume can be used.
Before applying a sample, the column should be equilibrated with 2 column volumes of buffer until a stable baseline is reached. Re-equilibration between runs is normally not necessary.
In the case that denatured proteins or lipids are not eluted within one column volume, the cleaning-in- place procedures should ensure the removal of these substances.

4. Maintenance

Depending on the individual applications, the media may be used many times. For the re-use purpose, please see the following instructions.

Cleaning-in-place (CIP)

CIP is a procedure that removes strongly bound materials such as lipids, endotoxins and denatured proteins that remain in the surface of the medium. 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 methods work as a general guidance.

Method 1 - 0.5 M NaOH solution at a linear flow velocity of roughly 15-25 cm/hour, at a reversed flow direction and with a contact time of 1-2 hours, can be used to clean impurities bound to the medium.

Method 2 - Apply two bed volumes of 0.1–0.5% detergent in a basic or acidic solution at a linear flow velocity of roughly 15-25 cm/hour, at a reversed flow direction. Residual detergent should be removed by washing the column with five bed volumes of concentrated organic solvent.

Method 3 - Apply a concentrated organic solvent such as two bed volumes of 70% ethanol or 30% isopropanol at a linear flow velocity of roughly 15-25 cm/hour, at a reversed flow direction. To avoid the formation of air bubbles, organic solvents should be applied in increasing concentration gradients.

For all methods, after the CIP step, the column should be equilibrated with at least 3 column volumes of buffer before the next run.

Sanitization
Sanitization using at least 1 bed volume of 0.5-1.0 M NaOH, at a flow velocity of 15-25 cm/hour is recommended. Following sanitization, the column should be re-equilibrated with 3-5 bed volumes of buffer.

5. Storage
The media should be stored in 20% ethanol (long term) or 0.02% sodium azide (short term) to prevent microbial growth. Store the media at a temperature of +2°C to +8°C.