ProteoSEC 6-5000 KDa

NB-45-0256-6-5000
NB-45-0257-6-5000
NB-45-0259-6-5000

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#Cat: NB-45-0256-6-5000 Size: 1each
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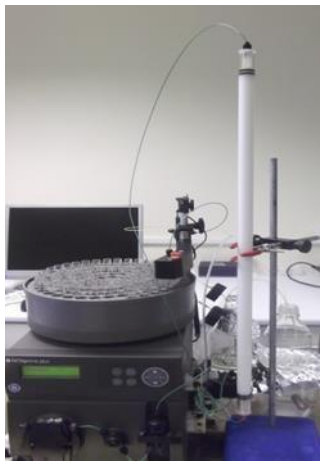
1. Introduction



Proteo SEC 6-5000KDa prep grade is a high-performance gel filtration medium specially designed for preparative purification of biological molecules having a large range of molecular weights (up to 5 million Dalton). Gel Filtration is a proven technique, which is widely used for size-based molecular separation.

Proteo SEC 6-5000KDa prep grade is a cross-linked, agarose-based medium optimised for high performance gel filtration of large biomolecules. The size and the distribution of the particles allow high efficiency, good flow and good capacity.

Ionic interactions: Eluent ionic strength of above 0.1 M is recommended.
Hydrophobic interactions: Some hydrophohic interactions have been recognized, i.e. some compounds may be eluted later than predicted. These interactions can be explored to achieve better resolution.

Characteristics of Proteo SEC 6-5000KDa Gel Filtration Medium:

Matrix	**Highly cross-linked agarose**
Optimal separation range (Mr)	6000 – 5 x 10^6
Particle size (µm)	Around 30 µm (in the range of 20 – 40 µm)
Operational pressure	Typically, 3 to 4 bar (0.3 – 0.4 MPa, 42 – 56 psi)
Operating flow velocity	<40 cm/hour (reducing the velocity in the condition of using solvents, caustics, viscous feedstock or low temperature)
pH stability	1-14 (short term cleaning) 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, 6 guanidine hydrochloride, 8 M urea, 30% isopropanol
Avoid	Oxidizing agents
Storage	20% ethanol

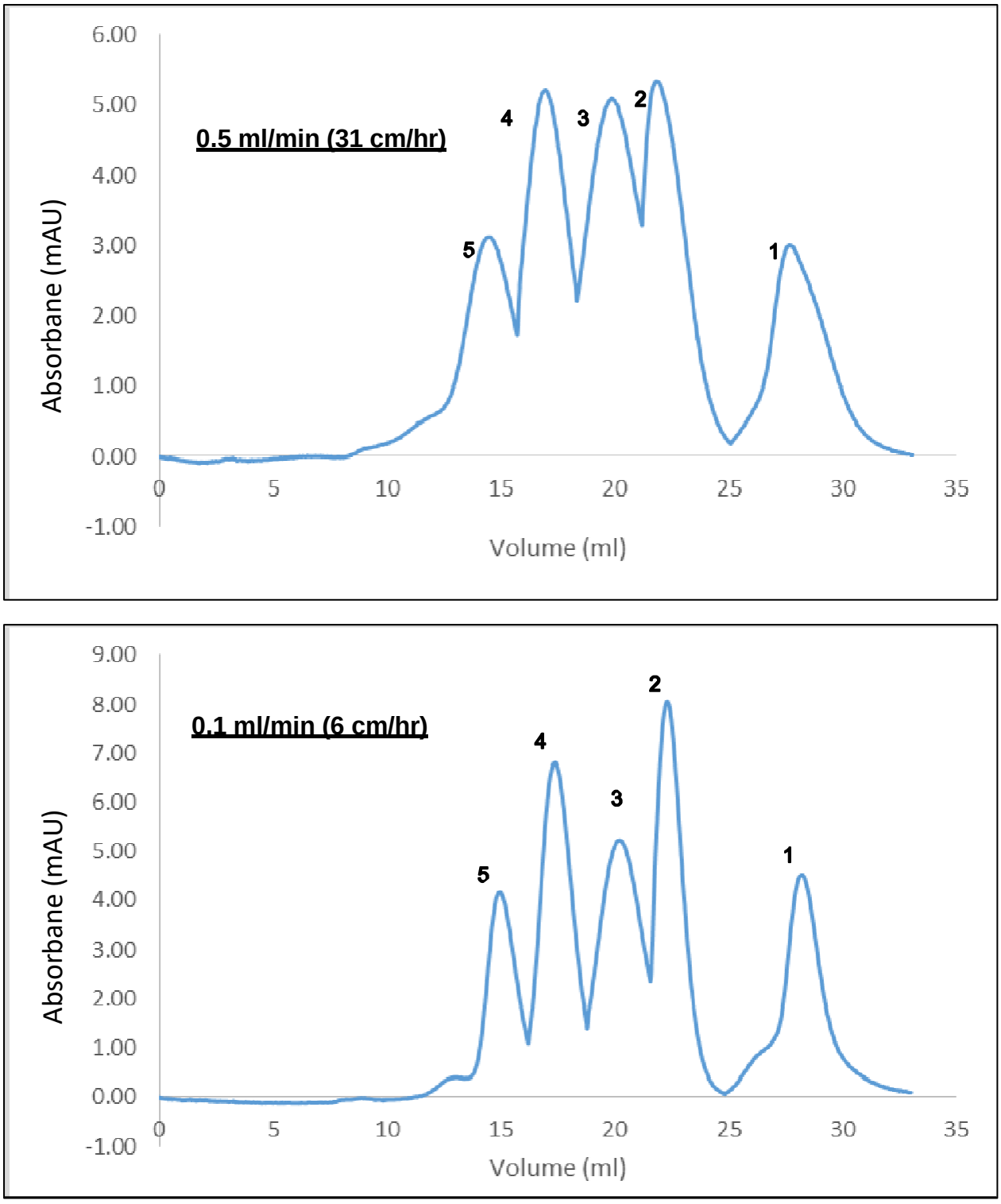


Figure 1: 11/30 gel filtration column packed with Poteo SEC 6-5000KDa medium run at different flow rates. Sample loading 0.1 ml; mobile phase: PBS (phosphate buffered saline); model proteins 1: aprotinin (Mr 6500); 2: β-lactoglobulin (Mr 35000); 3: ɣ-globulin IgG (Mr 158000; 4: apoferritin (Mr 440000); 5: thyroglobulin (Mr 669000)

Pre-packed Gel Filtration column specifications:

	Proteo SEC 11/30 / 16/60 & 26/60 6-5000
Matrix	**Highly cross-linked agarose**
Column hardware	Acrylic (column body); polypropylene and polyamide (plunger); NBR (o-ring)
Particle size (µm)	Around 30 µm (in the range of 20 – 40 µm)
Column body max pressure (*)	6 Bar, 0.6 MPa
Operating flow velocity	<40 cm/hour 0.1 – 0.6 ml/min Reducing the velocity in the condition of using solvents, caustics, viscous feedstock or low temperature
pH stability	1 – 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, 20% ethanol (Concentration of alcohol should not exceed 30% v/v)
Avoid	Oxidizing agents
Storage	20% ethanol or 10 mM sodium hydroxide

* Max resin pressure is 4 Bar.

2. Method optimisation

Eluent and sample preparation

The salt concentration of the eluent should preferably be at least 0.05 M to avoid ionic interactions. Choose an eluent providing good solubility for your sample. Water should be distilled water quality. Use analytical grade solvents, salts, and buffers.

Degas and filter all solutions through a 0.22 µm filter. Freshly filter samples through a 0.22 µm filter before loading to a column. Be sure to select a solvent resistant filter if samples are dissolved in organic solvents. If your sample is of high viscosity, dilute it with the eluent before applying to a column.

Column equilibration

Before applying the sample, equilibrate the column with at least three column volumes of eluent buffer. Longer equilibration may be needed with detergent solutions. Equilibrations is not needed between runs if the same eluent is used.

Flow rate optimisation

Good separations are generally obtained with low flow rates or longer elution times (e.g. 5 cm/h or less for 11/30 column). Large proteins and protein complexes often require overnight runs.

Loading capacity

Proteo SEC 11/30 column: up to 0.5 ml containing 25 mg protein or more if resolution is adequate.

3. Proteo SEC 6-5000KDa pre-packed column: Instruction of Use

Each packed column is sealed at both ends by mildly pressurised syringes filled with 20% ethanol.

1. Carefully remove all the packaging materials and place the bagged column on a flat surface. Always hold the middle part of the column when handling. **Never hold the column by the syringe side.** Cut the plastic bag and slide the column out of the bag.
2. Following the orientation labelling, firmly clamp the column at two points in a vertical position using a suitable support.

Disconnect the top syringe first.

3. Gently unhook the springs from the top side of the syringe using balanced force.
4. Unscrew the knob (located immediately above the top column plunger) from the column top to remove the sealing tubing / syringe system. Keep this syringe-tubing system for future use.
5. Connect the column top to the chromatography system. The column has female 1/16" thread connection. If M6 tubing connection is used, a 1/16" male thread – M6 female thread connector is required. It is recommended to have a flow rate ~0.5 ml/min and to fill the thread cavity with liquid before tightening the connection in order to avoid air bubbles.
6. Gently unhook the springs from the shaft of the bottom syringe using balanced force.
7. Unscrew the knob (located immediately below the bottom column plunger) to remove the sealing tubing / syringe system. Keep this syringe-tubing system for future use.
8. Connect the bottom of the column to the chromatography system.
9. Run the equilibration buffer to displace the storage buffer using a flow rate of up to 0.5 ml/min for 11 mm column, 1 ml/min for 16 mm column and 2.5 ml/min for 26 mm column. It is recommended to run through at least 3 column volumes of equilibration buffer before sample loading.

4. Column packing

End users can pack their own columns with Proteo SEC 6-5000KDa loose medium. 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 is 0.6 to 0.8 MPa. 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 2 mm below the marked level.
8. Test the packed column using tracer in water. Typically, 1% acetone with injection volume of 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.

5. Maintenance

Clean your column when you notice the column shows increased back pressure, or colour appears on the top part of the column or the resolution is obviously reduced.

Cleaning-in-place (CIP)

CIP is a procedure that removes strongly bound materials 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 method work as a general guidance.

Wash with 0.1 M to 0.2 M NaOH solution: Introduce 1/3 to 1 column volume of the NaOH solution at a linear flow velocity of roughly 15-25 cm/hour, then wash with deionised water. Re-equilibrate the column with plenty buffer and check pH.

6. Storage

The loose medium 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. The following is the guidance for storing a used column.

1. Run through at least 1.5 column volumes of 20% ethanol at a linear flow velocity of ≤30 cm/hr.
2. Suck 20% ethanol into each storage syringe tubing system until the syringe is fully filled. Invert each syringe and push air bubbles out of the tubing. Make sure the liquid volume inside the syringe is around 3-4 ml.
3. Disconnect the bottom of the column from the control system. Clamp the column upside down. Keep the pump running at a flow rate ≤0.5 mL/min until the thread cavity of the column is filled with 20% ethanol. Pause the pump. Screw one storage syringe to the column making sure that no air bubble is trapped.
4. Invert the column back to normal position. Start the pump. Disconnect the top of the column from the chromatography system and fill the top cavity of the column with 20% ethanol. Stop the pump.
5. Screw the other storage syringe tubing system into the top of the column making sure that no air bubble is trapped.
6. Carefully hold each syringe with one hand and hook the springs with the other hand to the shaft top of the syringe.
7. Place the column inside a cold room for long term storage.