Superose 6 prep grade
Superose 12 prep grade

Superose™ 6 prep grade (Code No). 17-0489-01) and Superose 12 prep grade (Code No. 17-0536-01) are high performance gel filtration media specially designed for preparative purification of biomolecules.

Introduction

Superose prep grade is a complement to prepacked columns of Superose 6 and 12. Superose prep grade can be efficiently packed under medium pressure in e.g. an HR 16/50 column for preparative work.

The instructions will help you to achieve the best results.

Contents of package

The package contains 125 ml of Superose prep grade in 20 % ethanol.

Media properties

Superose prep grade is a cross-linked, agarose-based medium optimised for high performance gel filtration of biomolecules. The size and the distribution of the particles alllow high flow, high efficiency and good capacity.

Properties

	Superose 6 prep grade	**Superose 12 prep grade**
Exclusion limit globular proteins (M_r)	4×10^7	2×10^6
Optimal separation range (M_r)	5 000–5×10^6	1 000–3×10^5
Matrix composition	Composite of cross-linked agarose	
Average Particle size (µm)	30 +-10 µm	30 +-10 µm
Max back pressure	0.4 MPa, 4 bar 60 psi	0.7 MPa, 7 bar 105 psi
recommended flow rate*	up to 40 cm/h	up to 40 cm/h
pH stability normal use	3–12	3–12
cleaning	1–14	1–14
Yields and activity recovery (%)	80–100	80–100

* At room temperature in aqueous buffer, If the column is used at +4 C half the flow rate compare to room temperature.

Ionic interactions: Negligble at an eluent ionic strength of above 0.05 M.

Hydrophobic interactions: Some hydrophohic interactions have been recognized, i.e. some compounds may be eluted later that predicted. These interactions can be of considerable value to the resolution.

Chemical and physical stability

Superose prep grade is resistant to all solutions commonly used in gel filtration, including 8 M urea, 6 M guanidine HCl and 30 % acetronitrile. A packed column should be used in the temperature range 4–40 ºC. The loose medium can he repeatedly autoclaved at pH 7, 120 ºC.

Superose prep grade is stable for long periods in the pH range 3–12 and for short periods, such as cleaning, in the pH range 1–14. High concentrations of formic acid should not be used. Superose 12 prep grande can be used in 0.1 M HCl. All detergents, non-ionic or ionic, such as SDS, may be used.Limited degradation of the polysaccharide chains may occur under oxidizing conditions.

Superose prep grade 6 can stand back-pressures up to 0.4 MPa (4 bar, 60 psi) and Superose 12 prep grade up to 0.7 MPa (7 bar, 105 psi).

Column packing and testing

Equipment

ÄKTAdesign pump or system

Packing Equipment HR 16 and Column HR 16/50 (~100 ml medium bed)

The HR 16/50 column is most suitable for high performance gel filtration since it is pressure stable.

Eluent

Destilled water or buffer solution.

To prepare the slurry

Wash the medium with destilled water in a glass filter (porosity less than 10 µm) to remove all ethanol.

The volume of the filtered medium is easy to measure on the glass filter.

HR 16/50-Suspend approximately 115 ml filtered medium in eluent to make 175 ml total suspension. To improve column packing, the suspension should contain 0.1 % Tween™ 20.

The suspension must be homogeneous and free from aggregates and should be degassed before packing.

Packing

Pack your column at the temperature it will be used at.

1. Make sure the filters are free from damage and that all parts are clean.
2. Assemble the column with a wetted bottom filter. Drop the plunger (part of the Filter tool supplied with the HR 16/50 column) into the HR 16/50 column with the large diameter end first. Shake the plunger up and down a few times so that the plunger pushes the bottom filter into place. Remove the plunger.
3. Attach the Packing Connector HR 16 to the column and connect an additional HR16/50 column (packing reservoir) to the other side of the packing connecter. Mount vertically the column with packing reservoir on a stand. Close the outlet tubing.
4. Wet the tube with eluent and pour the homogeneous medium suspension down the inside wall of the column. Pour all the medium in one operation. Fill the packing reservoir to the top with eluent.
5. Assemble and fasten the top of the packing reservoir with a top adaptor and connect it to the valve (pump). Open the outlet.
6. Pack the column in two steps. Recommended flow rates are given in the following table.

	HR16/50	
	Step 1	**Step 2**
Superose 6 prep grade	2 ml/min	3 ml/min
Superose 12 prep grade	2 ml/min	6 ml/min

   Allow all the medium to settle during step 1. Change directly to the flow rate recommended for step 2 and continue to pack for ~60 min. Do not exceed the pressure limits of the media.
7. Stop the pump and disconnect the packing reservoir on the HR 16/50 column.
8. Adjust the bed height of the HR 16/50 column to 510 mm. You can suspend excess medium by rotating the plunger supplied with the Filter Kit and remove it with a Pasteur-pipette (see Fig. 2 in the Filter Kit instructions).
9. Place a filter on the top of the medium bed in the HR 16/50 column. Follow the instructions supplied with the Filter Kit.
10. Insert and adjust the adaptor to the medium surface.
11. Connect the column inlet tubing to the valve (pump). Pack for 5–6 minutes with a flow rate giving the following back-pressures:

	AP
1. Superose 6 prep grade HR 16/50	0.4 MPa
2. Superose 12 prep grade HR 16/50	0.7 MPa

12. Disconnect the inlet tubing from the valve (pump) and adjust the adaptor to the medium surface and a further 2 mm down.

Testing

Test your column regarding efficiency (plate number, N, per metre). Use a sample of acetone, 5 mg/ml, in destilled water or buffer solution.

Test conditions

	HR 16/50
Sample volume:	200 µl
Flow rate:	1 ml/min
Detection:	280 nm

Calculate the plate number using the formula:

$$N = 5.54 \left(\frac{Vr}{W_{1/2}}\right)^2 \frac{1000}{L}$$

where

N = Number of theoretical plates per meter
Vr = Retention volume (ml)
$W_{1/2}$ = Peak width (ml) at half peak height
L = Bed length (mm)

Your column should have a plate number of at least 10 000 per metre to give satisfactory results. (15 000 plates/m may be possible to achieve.)

Connection the column to your system

1. Connect the shorter pre-flanged tubing (the outlet) to the detector.
2. For connection of the column to ÄKTAdesign system, you need two fingertight unions 1/16" male/M6 female (ÄKTA compatible). Connect the longer pre-flanged tubing (the inlet) to a injection valve.

Eluent and sample preparation

Degas and filter all solutions through a 0.22 µm filter. Water should be destilled water quality. Use analytical grade solvents, salts, and buffers. Centrifuge (10 000 × G for 10 minutes) or filter samples through a 0.22 µm filter. 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.

Column equilibration

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

Sample application

Make sure the sample is recently filtered or centrifuged before applying it to the column.

Sample elution

Flow rates above 25 cm/h are not recommended. The salt concentration of the eluent should preferably be at least 0.05 M to avoid ionic interactions.

Running conditions for best results

Flow rate optimisation

Very good separations are generally obtained 3–5 hours on Superose 12 prep grade and within 4–6 hours on Superose 6 prep grade (10–12 cm/h).

If you want to achieve the same results on Superose prep grade in an HR 16/50 column as on Superose 10/300 GL columns, select a flow rate giving approximately five times longer separation time on the bigger column. Large proteins and protein complexes often require overnight runs.

Loading capacity

HR 16/50: up to 2 ml containing 100 mg protein or more if resolution is adequate.

Eluent system

Choose an eluent providing good solubility for your sample. The salt concentration should generaly be at least 0.05 M to avoid ionic interactions.

Two columns in series

Coupling two columns in series increases the resolution by approximately 40 %. For connection, use the Union M6 female/M6 female (Code No.18-3856-01).

Column and medium cleaning

Clean your column if you observe:

- increased back-pressure
- colour change at the top of the column
- loss of resolution

Cleaning the packed column

Do not reverse the flow during cleaning since this may cause a loss of efficiency.

1. Change the filter on the top of the medium bed (HR 16/50) following the instructions supplied with the Filter Kit HR 16 (see "Spare parts and accessories").
2. Wash with 0.1 M-0.2 M NaOH: Turn the column up side down, inject at least 1/3 of the column volume and then elute with destilled water.
3. Equilibrate before your next run, check pH, and turn the column back to normal position.

Cleaning before repacking

1. Remove the bottom piece and empty the column by pumping destilled water or buffer through it. Clean all column parts with soapy water or laboratory detergents. Change the filters in the HR 16/50 column.
2. Wash the medium on a glass filter with 0.1–0.2 M NaOH, water and 20 % ethanol. Suspend the medium in destilled water (at least 5-fold the volume of medium) i a beaker. Let it sediment and pour off the supernatant. Repeat this washing once more before making the packing slurry.

Storage

Store your column in water or buffer containing 20 % ethanol. Avoid prolonged exposures to extremes of pH.

Spare parts and accessories

Designation	Quantity	Code No.
Column HR 16/50	1	18-1460-01
Packing Equipment HR 16	1	18-1442-01
Filter Kit HR 16	10	18-3585-01
Filter tool	1	18-3590-01
Capillary tubing (o.d- 1-8 mm, i-d- 0-5 mm)	2 m	19-7477-01
Tubing connectors*	5	19-7476-01
Flanging/Start-Up Kit		
120 V	1	19-5079-01
220 V	1	19-5090-01
Sample loops 1 ml, 2 ml	1 of each	18-5897-01
Superloop™ 10 ml	1	19-7585-01
Superloop 50 ml	1	19-7850-01
Superloop	1	18-1023-83
Solvent resistant O-ring (for the Superloop)	1	18-6300-01
SRTC-2, M6 female (0.5 mm i.d.). 2 HR columns in series	1	18-3856-01
Union, M6 female/1/16" female, stainless steel		
(Waters** compatible)	1	18-3405-01
(Swagelok™ compatible)	1	18-3406-01
Union- M6 female/1/16" female, titanium (Valco™ compatible)	1	18-3859-01
Union, M6 female/1/16" Male, plastic ÄKTA compatible	1	18-1112-58

* You need the Flanging/Start-Up Kit to attach new tubing connectors.

** Waters is our abbreviation for the fitting produced by Millipore Corp. Swagelok is a registered trademark of the Crawford Fitting Company. Valco is a trademark of Valco Instrument Co. Inc

Further Information

Visit www.amershambiosciences.com for more information.

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