Superdex 75 HR 10/30

INSTRUCTIONS

Superdex™ 75 HR/10/30 is a prepacked column for high performance gel filtration of proteins, peptides and other biomolecules. The column combines the superior separation properties of Superdex 75 with the advantages of an optimally packed high performance column.

Unpacking

Please check the delivery against this list.

Designation	Code No	No. supplied
Superdex 75 HR 10/30	17-1047-01	1
Wrench	19-7481-01	1
Filter Kit HR 10	18-3575-01	1
Filter tool	18-3590-01	1
Instructions		

Column description

The HR 10/30 column has an internal diameter of 10 mm. The height of the packed bed is 30 - 31 cm. The total bed volume is approx. 24 ml. Superdex 75 HR 10/30 is packed to the highest standards by GE Healthcare and each column is carefully tested regarding number of theoretical plates per metre.

The matrix, Superdex 75, is produced by the covalent bonding of dextran to highly cross-linked porous agarose beads. The separation properties of the composite medium is predominantly determined by the dextran component. The steep selectivity curve gives excellent resolution of proteins and peptides in the molecular weight range 3 000-70 000 Mr. Superdex 75 HR 10/30 is chemically stable over the pH range pH 3-12 during normal use and over the pH range pH 1-14 for cleaning. The principal properties of Superdex 75 HR 10/30 are given in Table 1.

Quality control test

To guarantee the quality of Superdex 75 HR 10/30, each column passes through an efficiency test, which shows that it has an approved theoretical plate number per metre (H-1) of min. 30 000. Each media batch undergoes a function test to ensure reproducible results

Connecting the column to ÄKTAdesign Systems

The column is delivered with a rubber tubing, connecting the outlet and inlet. The column is equilibrated in ethanol at a concentration of 20% on delivery.

1. Before connecting the column, start the pump and remove all air in your system, in particular in tubings and valves. Stop the pump.
2. Mount the column vertically in a column holder, remove the rubber tubing and connectors.
3. Connect the shorter preflanged tubing (the outlet) to the detector or to the lower column selector valve V2 using union FPLC™ female/HPLC male.
4. Connect the longer preflanged tubing (the inlet) to a valve for sample injection and elution or connect the column to the upper column selection valve V3.

Connecting the column to FPLC or HPLC systems

Superdex 75 HR 10/30 can be used with any chromatography system if the pump can provide precise and accurate flow within both the flow rate and pressure range of the column. For use with FPLC system no unions are required for connections. For use with HPLC systems the column should be connected as described for ÄKTA™design System via two unions which adapt the M6 connector to 1/16" tubing (see “Spare parts and accessories”).

Important before use

Column equilibration

- On delivery, the column is equilibrated with ethanol at a concentration of 20% as a bacteriostat. Wash out the ethanol using two column volumes (50 ml) of dist H_2O followed by two column volumes of equilibration buffer at a low flow rate (0.5 ml/min), keeping the back pressure below 1.8 MPa. Due to the viscosity of 20 % ethanol, a low flow rate is necessary to keep the back pressure below the recommended maximum (1.8 MPa).
- Equilibrate the column with another two column volumes of equilibration buffer.

Your column is now ready for use.

Chemical and physical stability

Superdex 75 HR 10/30 can be used with all aqueous buffer solutions commonly used in gel filtration over the pH range pH 3-12. Dissociating agents such as 6 M guanidine HCl, polar organic solvents such as 20%

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acetonitrile and detergents at concentrations normally used in chromatography can be used. Limited degradation of the polysaccaride chains may occur under oxidizing conditions.

The high rigidity of Superdex 75 allows the use of high flow rates. Excellent results have consistently been obtained in our laboratories with Superdex 75 HR 10/30 using flow rates of 1 ml/min (76 cm/h). The back-pressure should not be allowed to exceed **1.8 MPa**.

The column materials are all biocompatible and are therefore not limiting in the recovery of biological activity.

Column operation

Buffer preparation

To ensure long column life and trouble-free operation of Superdex 75 HR 10/30, care should be taken in preparing both buffers and samples. When preparing buffers use water and chemicals of high purity. Water should be of Milli-Q™ or corresponding quality. Use HPLC grade solvents, salts and buffers. Degas and filter all solutions through a 0.22 µm filter. Be sure to select a solvent resistant filter if the buffer contains an organic solvent.

As with all gel filtration matrices, certain pH dependent non-specific interactions can occur with both acidic and basic proteins at very low salt concentrations. It has also been observed that very hydrophobic peptides may be retained on the column to varying degrees. Deviations from "ideal" gel filtration of proteins and peptides on Superdex 75 can be reduced by using buffers with a salt concentration of at least 0.15 M, and by using polar organic solvents such as acetonitrile at a concentration of 20-30% if necessary when separating very hydrophobic peptides.

The elution buffer for the gel filtration step should ideally be chosen to simplify a later stage, e.g. lyophilization or another purification step.

Table 1. Properties of Superdex 75 HR 10/30.

Property	Description
Matrix	
*Exclusion limit (globular proteins)	100 000 Mr (approx.)
Optimal separation range (globular proteins)	3 000 - 70 000 Mr
Matrix composition	Composite of cross-linked agarose and dextran
Nominal bead size	13 µm
Prepacked column	
Bed dimensions	10 x 300-310 mm
Bed volume	24 ml (approx.)
Max. back pressure	1.8 MPa, 18 bar, 260 psi
Rec. flow rate	0.5 - 1.0 ml/min
**Max. flow rate	1.5 ml/min (110 cm/h)
Column efficiency (N)	> 30 000/m
pH stability (long term)	3-12
pH stability (short term)	1-14

* Exclusion limit is calculated by extrapolation of the linear part of the selectivity curve. Practically, proteins with a molecular weight greater than 200 000 MW will be excluded from the matrix.

** At room temperature in aqueous buffer. Flow rate is determind by $v \cdot \eta \leq 1.5$ where V = flow rate and η = viscosity. Column life is optimised when used within the recommended flow rate range.

Sample preparation

Centrifuge (10 000 x g for 10 min) or filter samples through a 0.22 µm - 0.45 µm filter. Be sure to select a solvent resistant filter if samples are dissolved in organic solvents.

Column equilibration

Before applying the sample, equilibrate the column with two column volumes (50 ml) of elution buffer.

Sample application and elution

Ensure the sample has been prepared according to the recommendations given above.

For highest resolution, sample volumes of between 0.1-1.0% (25-250µl) of the bed volume (Vc) are recommended. Normally, relatively high sample concentrations can be used in gel filtration without significantly compromising resolution. This should be determined on a case-by-case basis.

If the sample is of high viscosity, dilute with elution buffer so that the maximum back-pressure (**1.8 MPa**) is not exceeded during sample application. Otherwise use lower flow rates.

The most convenient and reproducible method of sample injection is via the valves INV-907, V-1,V-7, PV-7, MV-7 or PMV-7. Large volumes can be applied using a Superloop (see "Spare parts and accessories").

Separations by gel filtration are best optimised by starting with a high flow rate and a relatively low sample volume. A starting flow rate of 1.0 ml/min (76 cm/h) and a sample volume of 0.1% Vc is recommended. Depending on the difficulty of the separation, both flow rate and sample volume should then be optimized to give the required resolution in as short a cycle time as possible. Optimal resolution should be expected with flow rates of 0.5 - 1.0 ml/min (38-76 cm/h). Large molecules often require lower flow rates, 0.1-0.5 ml/min, for maximal resolution.

Two columns in series

Resolution in gel filtration can be increased by increasing the total bed height. The bed height of Superdex 75 HR 10/30 can be doubled by connecting two columns in series using the Union, M6 female/M6 female, Code No. 18-3856-01. **The total back pressure should not be allowed to exceed 3 MPa.**

Molecular weight determinations

Calibration of Superdex 75 HR 10/30 allows the estimation of protein molecular weights. Apply suitable sample proteins with known molecular weights to obtain a calibration curve. Use normal conditions, for example 0.05 M PBS, pH 7.0, or denaturing conditions, for example 6 M guanidine-HCl.

A recommended protein mixture is:

	Mol. wt.
Aldolase	158 000
BSA	67 000
Ovalbumin	43 000
Chymotrypsinogen A	25 000
Myoglobin	17 600
Ribonuclease A	13 700
Aprotinin	6 500
Vitamin B12	1 355

The void volume of the column can be determined using Blue Dextran 2000. The K_{av} for the individual proteins can be calculated as follows:

$K_{av} = (V_R - V_0)/(V_c - V_0)$
where
V_0 = void volume of the column
V_R = elution volume of the protein
V_C = the geometric bed volume in ml

Column cleaning

To maximize the life length of the column, regular column cleaning is recommended. A recommended general cleaning method is one column volume (25 ml) of 0.5 M NaOH at 0.5 ml/min. The frequency of cleaning is of course dependent on the degree of contamination, but a cleaning cycle at least every 10-20 separation cycles is recommended.

After cleaning, immediately equilibrate the column with at least two bed volumes of equilibration buffer. Before applying sample, the UV baseline should be stable and the pH must be checked.

If you suspect the column to be still contaminated, refer to the "Trouble shooting" section below.

Trouble-shooting

If the column registers an increased back-pressure, if the gel is discoloured, if a space develops between the adaptor and the gel bed, if there is a loss of resolution or if you otherwise suspect the column to be contaminated, follow the procedures below.

- Change the top filter. Instructions for changing the filter are supplied with the Filter Kit.
- Perform a more rigorous column cleaning procedure. 1 M NaOH and 0.1 M HCl can be used as cleaning agents with Superdex 75 HR 10/30. Studies carried out in GE Healthcare have shown that the separation properties of Superdex 75 are not significantly affected after twenty five two-hour cleaning cycles using these solvents.
- If air enters the column, remove using equilibration buffer at 0.5 ml/min. The quality of the packed bed will not normally be affected.

Efficiency test

After column maintenance procedures the efficiency of the column should be checked. Column efficiency, expressed as plates per metre (H-1), is estimated using the following equation:

$H^{-1} = 5.54 \times (V_R/w_h)^2 \times (1\,000/L)$

L = bed height (mm)
V_R = peak retention (elution) volume (ml)
w_h = peak width at half peak height (ml)
H^{-1} = number of theoretical plates/metre

Sample: 100 µl of acetone (p.a.), 5.0 mg/ml

Eluent: 20% (v/v) ethanol
Flow rate: 0.8 ml/min
Detector: UV-M, 280 nm, 0.01 AUFS
Chart speed: 3 cm/min

Function test

An alternative to the efficiancy test to check column performance is the function test described here.
Experimental:

Sample: 100 µl solution containing
1. Transferrin, 2 mg/ml
2. Ovalbumin, 2.5 mg/ml
3. Myoglobin, 1 mg/ml
4. Ribonuclease A, 5 mg/ml
5. Aprotinin, 2 mg/ml

Buffer: 0.05 M phosphate buffer with 0.15 M NaCl, pH 7.0
Flow rate: 0.4 ml/min
Detector: UV- M, 280 nm, 0.5 AUFS
Chart speed: 0.5 cm/min

Column storage

If the column is to be stored for more than a couple of days, the column should be equilibrated in a buffer containing a suitable bacteriostat. Ethanol at a concentration of 20% is recommended: equilibrate with two column volumes of distilled H_2O followed by two column volumes of 20% ethanol before storage.

Connect the rubber tubing supplied with the column between the inlet and the outlet of the column. The tubing should be filled with the storage solution. This will prevent column drying.

The column may be stored at ambient temperature. We recommend 4 °C for long term storage.Further information.

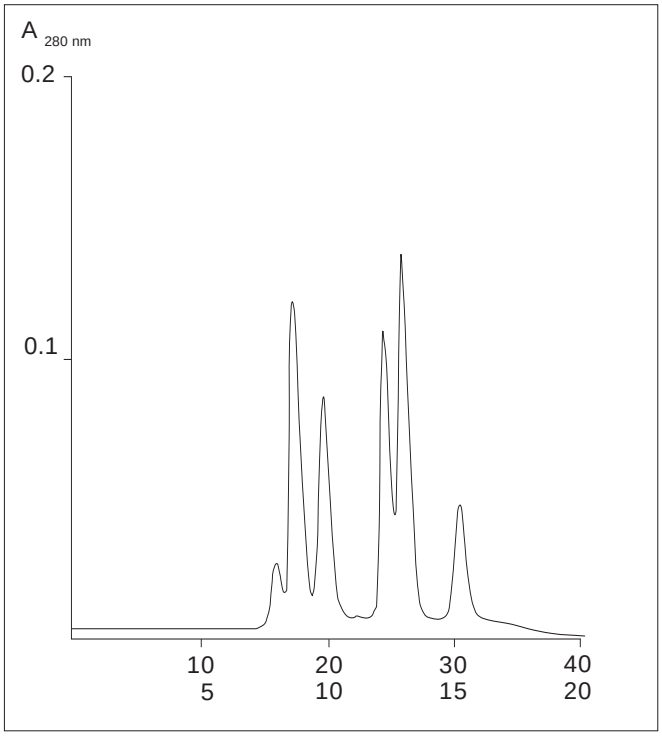

Fig. 1. Typical chromatogram from a function test of Superdex 75 HR 10/30.

Important Information

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For further information; please contact your local GE Healthcare representative.

Spare parts and accessories

Designation	Code No.	No. per pack
Top assembly HR 10	18-1541-01	1
Bottom assembly HR 10	18-1542-01	1
Filter Kit HR 10	18-3575-01	10
Filter tool	18-3590-01	1
Capillary tubing (o.d. 1.8 mm, i.d. 0.5 mm)	19-7477-01	2 m
Tubing connectors*	19-7476-01	5
Flanging/Start Up kit		
120 V	19-5079-01	1
220 V	19-5090-01	1
Prefilter	19-5084-01	1
Filters + O-rings (prefilter)	19-5082-01	5+2
Sample loops 1 ml, 2 ml	18-5897-01	1 of each
Superloop™ 10 ml	19-7585-01	1
Superloop 50 ml	19-7850-01	1
Solvent resistant O-ring (for the Superloop)	18-6300-01	1
Union FPLC female/HPLC male (i.d 0.8 mm), PEEK	18-1112-58	1
Union, M6 female/1/16" female, stainless steel		
(Waters™ compatible)	18-3405-01	2
(Swagelok™ compatible)	18-3406-01	2
Union M6 female/1/16" female, titanium		
(Valco™ compatible)	18-3859-01	1
Union, M6 female/1/16" male, plastic		
(Valco compatible)	18-3858-01	5
Domned nut, M6	18-2450-01	4

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

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