Superdex 200 HR 10/30

INSTRUCTIONS

Superdex™ 200 HR 10/30 is a prepacked column for high performance gel filtration of proteins, DNA fragments (<200 bp) and other biomolecules. The column combines the superior separation properties of Superdex 200 with the advantages of an optimally packed high performance column.

Unpacking

Please check the delivery against this list.

Designation	Code No	No. supplied
Superdex 200 HR 10/30	17-1088-01	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 approximately 24 ml. Superdex 200 HR 10/30 is packed to the highest standards by Amersham Biosciences and each column is carefully tested regarding number of theoretical plates per metre.

Superdex 200 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 10 000–600 000.

Superdex 200 HR 10/30 is chemically stable over the pH range 3–12 during normal use and over the pH range 1–14 for cleaning. The principal properties of Superdex 200 HR 10/30 are given in Table 1.

Quality control tests

To guarantee the quality of Superdex 200 HR 10/30, the efficiency of each column is tested. Each media batch undergoes a function test to ensure reproducible results.

Connecting the column to ÄKTA™design 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 selection 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 selection valve V3.

Connecting the column to FPLC™ or HPLC systems

Superdex 200 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”).

71-7059-00
Edition AE

Amersham Biosciences

Important before use

Column equilibration

- On delivery the column is equilibrated with 20% ethanol 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 1.5 MPa. Due to the viscosity of 20% ethanol a low flow rate is necessary to keep the back pressure below the recommended maximum (1.5 MPa).
- Equilibrate the column with another two column volumes of equilibration buffer.

Your column is now ready for use.

Chemical and physical stability

Superdex 200 HR 10/30 can be used with all aqueous buffer solutions commonly used in gel filtration over the pH range 3–12. Dissociating agents such as 6 M guanidine-HCl, polar organic solvents such as 30% 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 200 allows the use of high flow rates. Excellent results have been obtained using flow rates of 1 ml/min (76 cm/h). The back-pressure should not be allowed to exceed **1.5 MPa.**

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

Table 1. Properties of Superdex 200 HR 10/30.

Property		Description
Matrix		
Exclusion limit* (globular proteins)		1.3×10^6 M_r
Optimal separation range (globular proteins)		10 000–600 000 M_r
Matrix composition		Composite of cross-linked agarose and dextran
Nominal bead size		13 µm
Prepacked column		
Bed dimensions		10 × 300–310 mm
Bed volume		24 ml (approx.)
Max. back pressure		1.5 MPa, 15 bar, 217 psi
Max. flow rate**		1 ml/min
Rec. flow rate		0.25–0.75 ml/min
Column efficiency (H^{-1})		≥ 30 000
pH stability	(working range)	3–12
	(cleaning range)	1–14

* Exclusion limit is calculated by extrapolation of the linear part of the selectivity curve. Practically, proteins with a molecular weight greater than 1.3×10^6 will be excluded from the matrix.

** At room temperature in aqueous buffer. Flow rate is determined by $V \cdot \eta \leq 1.0$ where V = flow rate and η = viscosity. The column life is optimised when used within the recommended flow rate range.

Column operation

Buffer preparation

To ensure long column life and trouble-free operation of Superdex 200 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 MilliQ™ or corresponding quality. Use HPLC grade solvents, salts and buffers. Degas and filter all solutions through a 0.22–0.45 µm filter. Be sure to select a solvent resistant filter if the buffer contains an organic solvent.

A recommended buffer is:

50 mM Na-phosphate, pH 7.0 + 0.15 M NaCl

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. Therefore an ionic strength of ≥ 20 mS/cm is recommended. If necessary polar organic solvents such as acetonitrile at a concentration of up to 30% can be used.

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

Sample preparation

Centrifuge (10 000 × *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% (25–250 µl) of the bed volume (V_c) are recommended. However, at lower flow rates (≈ 0.25 ml/min) a sample volume of 500 µl is possible.

Relatively high sample concentrations of up to 40 mg/ml can be used without significantly compromising resolution. A maximum protein amount is around 10 mg, but it should be determined on a case-by-case basis.

If the sample is of high viscosity, dilute it with elution buffer so that the maximum back pressure (1.5 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 V-7, PV-7, MV-7, PMV-7 or INV-907.

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 ml/min (76 cm/h) and a sample volume of 25 µl ($0.1\% \times V_c$) are 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 0.25–0.75 ml/min depending on the sample. Large molecules often require lower flow rates 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 200 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 2.5 MPa.**

Molecular weight determinations

Calibration of Superdex 200 HR 10/30 allows the estimation of protein molecular weights to obtain a calibration curve. Use normal conditions, 50 mM Na-phosphate, pH 7.0 + 0.15 M NaCl, or denaturing conditions, where 6 M guanidine-HCl, 8 M urea or 0.1 % SDS has been added.

A recommended protein mixture for normal conditions is:

Sample	M_r	Conc (mg/ml)	Supplier
Thyroglobulin	669 000	3	Amersham Biosciences
Ferritin	440 000	0.7	Amersham Biosciences
Human IgG	160 000	3	Amersham Biosciences
Transferrin	81 000	3	Sigma T-0519
Ovalbumin	43 000	3.5	Sigma A-2512
Myoglobulin	17 600	1.5	Sigma M-0630
Vitamin B_{12}	1 355	0.5	Sigma V-2876
Sample volume:	250 µl		
Flow rate:	0.5 ml/min		

Recommended proteins for normal or denaturing conditions are:

Sample	M_r	Conc	Supplier (mg/ml)
Thyroglobulin	669 000 165 000*	≈ 2.5	Amersham Biosciences
Human IgG	150 000 50 000* 25 000*	≈ 3.0	Amersham Biosciences
Transferrin	81 000	≈ 2.0	Sigma T-0519
BSA	67 000	≈ 2.0	Sigma A-4503
Ovalbumin	43 000	≈ 2.0	Sigma A-2512
Ribonuclease A	13 700	≈ 2.0	Sigma R-5000
Cytochrome C	12 300	≈ 2.0	Sigma C-2506
Blue dextran		≈ 1.0	Amersham Biosciences
Sample volume:	100 µl		
Flow rate:	0.4 ml/min (buffer incl. 6 M guanidine-HCl or 8 M urea) 1.0 ml/min (buffer incl. 0.1% SDS)		

* Sub units

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:

$$K_{av} = (V_R - V_O)/(V_C - V_O)$$

where
V_O = void volume of the column
V_R = retention (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 0.1 M HCl or 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 after every 10–20 separation cycles is recommended.

Note: Do not leave the column in 0.5 M NaOH after the cleaning cycle as that may cause dextran leakage.

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.

For very hydrophobic proteins and lipids another cleaning method is suggested:

1. 5 ml of 70% ethanol or 30% acetonitrile, flow rate 0.2 ml/min.
2. 5–10 ml of water, flow rate 0.4 ml/min.
3. 5–10 ml of buffer, flow rate 0.4 ml/min.

If the column is still not restored, try injecting a solution of 1 mg/ml pepsin in 0.1 M acetic acid and 0.5 M NaCl and then leave it overnight at room temperature or for 1 hour at 37°C. Depending on the contamination other enzymes can be used, e.g. DNAse. After the enzymatic treatment, repeat the cleaning process.

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 200 HR 10/30. Do not leave the column in 1 M NaOH (see above).
- 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 mettre (H^{-1}), is estimated using following equation:

$H^{-1} = 5{,}54 * (V_R/w_h)^2 * (1\,000/L)$
L = bed height (mm)
V_R = peak retention (elution) volume (ml)
w_h = peak width at half peak hight (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: 0.75 ml/min
Detector: UV-M, 280 nm, 0.01 AUFS
Chart speed: 3 cm/min

Function test

An alternative to the efficiency test to check column performance is the function test described in Figure 1.

Sample: 100 µl solution containing
1. IgG (M_r 160 000), 2.5 mg/ml
2. BSA (M_r 67 000), 8 mg/ml
3. β-lactoglobulin (M_r 35 000), 2.5 mg/ml
4. Cytochrome C (M_r 12 400), 1 mg/ml
5. Vitamin B_{12} (M_r 1355), 0.1 mg/ml
6. Cytidine (M_r 243), 0.1 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

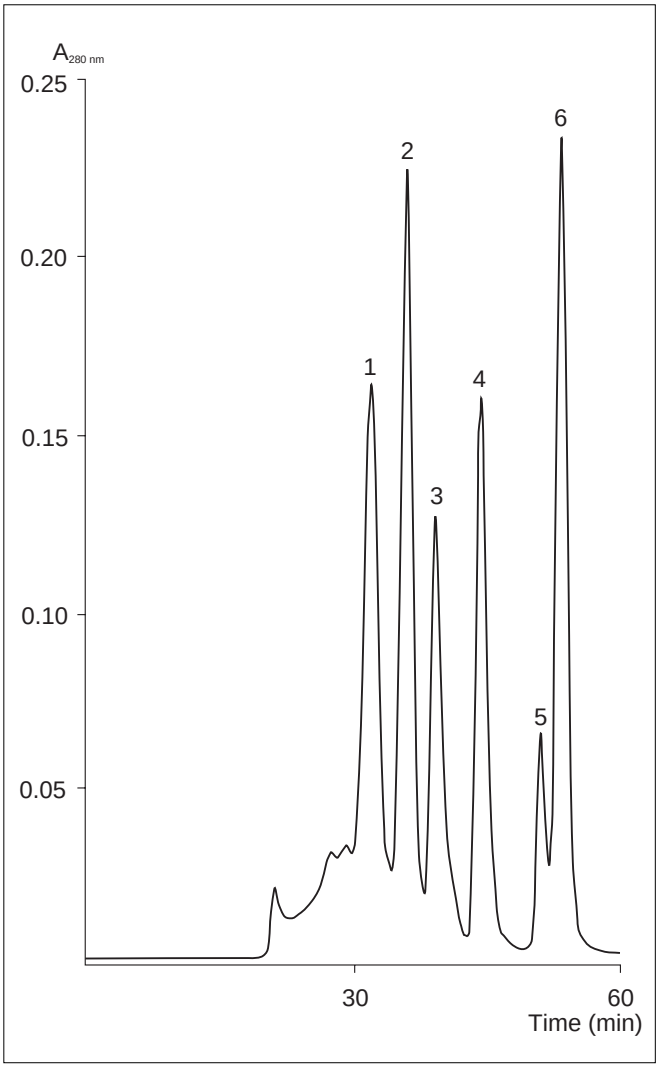

Fig. 1. Typical chromatogram from a function test of Superdex 200 HR 10/30.

Column storage

If the column is to be stored for more than a couple of days, the column should be equilibrated in 20% ethanol or a buffer containing a suitable bacteriostat. Equilibrate with two column volumes of dist 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

For further information, please contact your local Amersham Biosciences 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
Assorted sample loops	18-0404-01	
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
Superloop 150 ml	18-1023-83	1
Solvent resistant O-ring (for the Superloop)	18-6300-01	1
Union, FPLC female/ HPLC male (ID 0.8), PEEK	18-1112-58	
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.

Amersham Biosciences UK Limited Amersham Place Little Chalfont Buckinghamshire England HP7 9NA

Amersham Biosciences AB SE-751 84 Uppsala Sweden

Amersham Biosciences Inc 800 Centennial Avenue P.O. Box 1327 Piscataway NJ 08855 USA

Produced by Wikström, Sweden 982227, Dec., 1998
Printed matter. Licence 341 051