Superdex Peptide HR 10/30

INSTRUCTIONS

Superdex™ Peptide HR 10/30 is a pre-packed column for high performance size exclusion chromatography of natural, recombinant or synthetic peptides and other small biomolecules. The column combines the superior separation properties of Superdex Peptide with the advantages of an optimally packed high performance column.

Unpacking

Please check the delivery against this list.

Designation	Code No	No. supplied
Superdex Peptide HR 10/30	17-1453-01	1
Wrench	19-7481-01	1
Filter Kit HR 10	18-3575-01	10
Filter tool	18-3590-01	1
Union, M6 female/1/16″ male, plastic	18-3858-01	5
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 Peptide HR 10/30 is packed to the highest standards by Amersham Biosciences.

Superdex Peptide is produced by the covalent bonding of dextran to highly cross-linked porous agarose beads. The separation properties of the composite medium are predominantly determined by the dextran component. The steep selectivity curve gives excellent resolution of peptides in the molecular weight range 100–7 000.

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

Quality control tests

To guarantee the quality of Superdex Peptide 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 for protection during transport and storage. The column is equilibrated with 20% ethanol.

1. Before connecting the column, start the pump and remove all air from your system, in particular in tubing and valves. Stop the pump.
2. Mount the column vertically in a column holder. Remove the rubber tubing and connectors.
3. Connect the shorter pre-flanged tubing (the outlet) to the detector or to the lower column selection valve V2 using Union FPLC female/ HPLC male.
4. Connect the longer pre-flanged 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 Peptide HR 10/30 can be used with any chromatography system if the pump can provide precise and accurate flow within the flow rate and pressure range of the column. For use with FPLC system no unions are required for connection. For use with HPLC systems the column should be connected as described for ÄKTA design Systems with two unions which adapt the M6 connector to 1/16″ tubing (see “Spare parts and accessories”).

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Important before use

Column equilibration

- On delivery the column is equilibrated with 20% ethanol as a bacteriostat. Wash out the ethanol and equilibrate the column with 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). Ensure that the back pressure does not exceed 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.

Your column is now rady for use.

Chemical and physical stability

Superdex Peptide HR 10/30 can be used with all eluents commonly used in size exclusion chromatography over the pH range 1–14. Dissociating agents such as 6 M guanidine HCl, polar organic solvents such as 20–70% acetonitrile and detergents at concentrations normally used in chromatography can be used.

The high rigidity of Superdex Peptide 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 Peptide HR 10/30.

Property	Description
Matrix	
Exclusion limit[1]	20 000
Optimal separation range (peptides)	100–7 000
Matrix composition	Composoite of cross-linked agarose and dextran
Nominal bead size	13 mm
Pre-packed column	
Bed dimensions	10 × 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[2]	1.2 ml/min
Column efficiency (H^{-1})	≥ 30 000
pH stability (normal use)	1–14
(cleaning)	1–14

[1] Exclusion limit is calculated by extrapolation of the linear part of the selectivity curve. In practice, molecules with a molecular weight greater than 20 000 will be excluded from the matrix.

[2] At room temperature in aqueous buffer. Flow rate is determined by $V \times \eta \leq 1.2$ when V = flow rate and η = viscosity. 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 Peptide HR 10/30, care should be taken in preparing both eluents and samples. When preparing eluents, use water and chemicals of high purity. Water should be of Milli-Q™ or corresponding quality. Use HPLC grade solvents, salts and buffers. De-gas and filter all solutions through a 0.22 mm filter. Be sure to select a solvent resistant filter if the eluent contains an organic solvent.

Selecting the eluent

As with all size exclusion matrices, non-specific interactions can occur. Peptides and small biomolecules are more susceptible than larger proteins to interactions with the gel medium, as peptides display very little or no tertiary structure leaving the amino acid side chains more exposed. Small differences in shape also affect the retention. Proper selection of elution conditions is very important for peptide separations, to minimise any undesired secondary effects, such as ionic or hydrophobic interactions between the sample and the matrix. The buffer should ideally be chosen to simplify a later stage, e.g. lyophilization or another purification step. Some general guide lines are given below.

Volatile eluents

If the peptides are to be lyophilized, a volatile eluent is necessary. Acetonitrile (20–30%) in water with TFA (0.1%) is generally useful. Up to 70% acetonitrile may be used. Peptides which are extremely hydrophobic may be separated in 70% formic acid.

Aqueous buffer solutions

Standard aqueous buffer solutions, for example phospate buffer (0.02 M) containing NaCl (0.25 M), may also be used. Acidic peptides are best run at pH >8 to improve their solubility. Similarly, basic peptides can be separated at pH <7.

Sample preparation

Centrifuge (10 000 × g for 10 min) or filter samples through a 0.22 mm–0.45 mm 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 at least two column volumes (50 ml) of elution buffer.

Sample application and elution

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

Sample volumes of between 0.1–1.0% (25–250 ml) of the bed volume (V_c) are recommended for the best resolution.

Relatively high sample concentrations, up to 10 mg/ml in combination with a 250 ml loop, can be used without significantly compromising resolution. The maximum amount of sample which can be used depends on its complexity and the resolution which is required.

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 through the valves V-7, PV-7, MV-7, PMV-7 or INV-907.

Optimisation

Separations by size exclusion are best optimised by starting with a high flow rate and a relatively small sample volume. A starting flow rate of 1 ml/min (76 cm/h) and a sample volume of 25 ml (0.1% V_c) are recommended. Flow rate and sample volume can then be adjusted to give the required resolution in as short a cycle time as possible. Optimal resolution can be expected at a flow rate in the range 0.2–1 ml/min.

Two columns in series

Resolution in size exclusion chromatography can be increased by increasing the total bed height. The bed height of Superdex Peptide 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 for two columns in series should not be allowed to exceed 2.5 MPa.**

Column cleaning

Regular column cleaning is recommended to maximise the life of the column. How often the column needs to be cleaned will depend on the samples that are run. A regular cleaning cycle after every 10–20 separation cycles is used in our laboratories.

Regular cleaning cycle

1. Wash the column with 25 ml NaOH (0.5 M) or acetonitrile (30–70%) at a flow rate of 0.5 ml/min.
2. Equlibrate the column with at least two bed volumes of equilibration buffer. The UV baseline and pH should be stable before applying sample.

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

Trouble shooting

Air in the column

If air enters the column, it may usually be removed by running well de-gassed equilibration buffer through the column at 0.5 ml/min. The quality of the packed bed will not normally be affected.

Contaminated column

If the gel becomes discoloured, if a space develops between the adapter and the top of the gel bed, if resolution is lost or if you otherwise suspect the column to be contaminated, a more rigorous cleaning procedure may be necessary.

Increased back-pressure

More rigorous cleaning may also be needed if the back-pressure increases. However, before cleaning, make sure that the high back-pressure in the system is in fact caused by the column.

Disconnect one piece of equipment at a time, starting at the fraction collector, with the pumps working. Check the pressure reading after each piece is disconnected to determine the source of the blockage. A dirty prefilter is a frequent cause of increased back-pressure.

Check the back-pressure at the same stage during each run, since the back-pressure can vary within a run, e.g. injecting a sample and mixing different eluents may cause increased back-pressure.

Rigorous cleaning procedure

1. Change the filter at the top of the column. Instructions for changing the filter are supplied with the Filter kit. Since contaminants are introduced with the liquid flow, many of them are caught by the filter.
2. Fill the column with the cleaning agent and allow it to stand for 2 hours. Acetonitrile (70%), NaOH (1 M), acetic acid (1 M) and HCl (0.1 M) may be used.
3. Wash the cleaning agent out with water and equilibrate with the required eluent until the baseline is stable.

Note: Re-equilibration after cleaning with strong acid or base is faster if the column is washed with concentrated buffer solution before final equilibration with the eluent.

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 distance (mm)

w_h = peak width at half peak height (mm)

H^{-1} = number of theoretical plates/metre

Sample: 100 µl acetone, 5 mg/ml
Eluent: Milli-Q water
Flow rate: 1.0 ml/min
Detector: UV-M, 280 nm, 5 mm cell, 0.5 AUFS
Chart speed: 3 cm/min
System: FPLC System

Function test

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

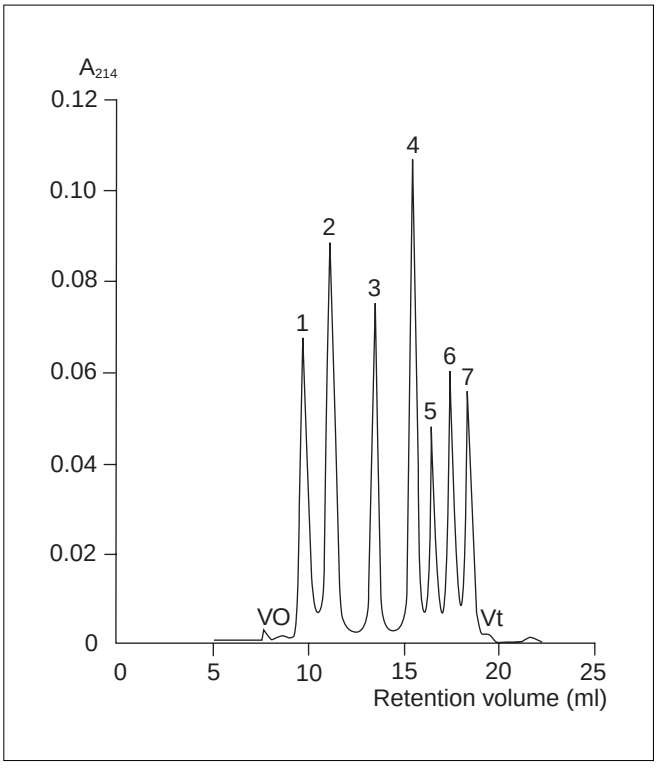

Fig. 1. Separation of peptide standards.

Expermental

Column: Superdex Peptide HR 10/30
Sample:*
1. Cytochrome C, 0.2 mg/ml, M_r 12 500
2. Aprotinin, 0.2 mg/ml, M_r 6 500
3. Gastrin I, 0.2 mg/ml, M_r 2 126
4. Substance P, 0.2 mg/ml, M_r 1 348
5. $(Glycine)_6$, 0.2 mg/ml, M_r 360
6. $(Glycine)_3$, 0.2 mg/ml, M_r 189
7. Glycine, 7.0 mg/ml, M_r 75

Sample volume: 25 ml
Buffer: 0.25 M NaCl in 0.02 phosphate buffer, pH 7.2
Flow rate: 0.25 ml/min
Detection: UV-M, 214 nm, 5 mm cell, 0.1 AUFS
Chart speed: 0.5 cm/min
System: FPLC System

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 columns volumes of 20% ethanol before storage.

Fill the rubber tubing supplied with the column with the storage solution and connect it between the inlet and the outlet of the column. This prevents the column from drying.

The column may be stored at ambient temperature. We recommend +4 ºC 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		
120V	19-5079-01	1
220V	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
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/M6 female	18-3856-01	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	1
Domed nut, M6	18-2450-01	4

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

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