

Fast Desalting Column HR 10/10

INSTRUCTIONS

Columns prepacked with Sephadex® G-25 Superfine offer fast desalting of samples up to 1 ml in volume.

Introduction

Fast Desalting Column HR 10/10 is designed to facilitate rapid desalting or buffer exchange e.g. before ion exchange and chromatofocusing or after ion exchange and hydrophobic interaction chromatography. A special high density packing makes the column highly efficient; desalting takes only 1-4 minutes. The column has a long life, and repeated use may be easily automated with FPLC® System.

Unpacking

The package contains:

Designation	Code No.	No. supplied
Fast Desalting Column HR 10/10	17-0591-01	1
Filter Kit HR 10 (10 filters + 4 O-rings)	18-3575-01	1
Filter tool	18-3590-01	1
Instructions		1

Connecting the column to FPLC System

1. The HR 10/10 column is supplied with rubber tubing connecting the inlet to the outlet of the column. Remove this tubing and the connectors, but keep them for future storage of the column.
2. Connect the shorter preflanged tubing (the outlet) to the detector or to a selection valve.
3. Connect the other end to a valve which can be positioned for sample injection and elution e.g. Amersham Biosciences Valve V-7 or Motor Valve MV-7. When using the Valve PV-7 or the Motor Valve PMV-7, connect the Union, M6 female/1/16" male (Code No. 18-3858-01) between the flanged tubing and the valve.

Important before use

The pre-packed column is pressure rated up to 1.2 MPa (12 bar, 170 psi). However, a precipitation of the sample may block the filter and cause it to compress the gel. To avoid this, we advise you to set the pressure limit control to only a few (2-3) bars over your actual operation pressure. The gel is supplied in 20% ethanol. Wash out the ethanol with at least five column volumes of water (Milli-Q*). Start with a low flow rate, 0.1 ml/min, and increase it gradually to 3 ml/min to avoid high back-pressures. Do not exceed 1 MPa (10 bar, 140 psi) during solvent change. After washing, equilibrate with at least two column volumes of buffer. We do not recommend flow rates above 6 ml/min.

Connecting the column to HPLC systems

You can use Fast Desalting Column HR 10/10 with any HPLC system if the pump can provide precise and accurate flow at relatively low back-pressures. Connect the column as described for FPLC System, but via two unions which adapt 1.8 mm to 1/16" tubing (see "Spare parts and accessories").

Gel properties

Sephadex G-25 Superfine is a beaded gel filtration matrix, based on cross-linked dextran, which exhibits high selectivity. The exclusion limit (M_r) for proteins and peptides is approximately 5 000, making Fast Desalting Column HR 10/10 useful in many separation schemes.

* Milli-Q is registered trademark of Millipore Corp.

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Edition AE



GE imagination at work

Chemical and physical stability

Fast Desalting Column HR 10/10 is stable in the pH range 2-13. The packed gel bed withstands up to approximately 1.2 MPa (12 bar, 170 psi) in back pressure while the HR 10/10 column itself can stand 5 MPa (50 bar, 715 psi). A normal back-pressure with pure water is approximately 0.7 MPa (7 bar, 100 psi) at 6 ml/min. During solvent change, do not exceed 1 MPa (10 bar, 140 psi).

Note: A compressed gel bed cannot be restored if the bead structure is destroyed and has to be repacked.

Eluent and sample preparation

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 sterile filter. Either centrifuge (10 000g for 10 min) or filter samples through a 0.22 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 under the chosen conditions until the base-line is stable.

Sample application

Make sure that the sample is recently filtered or centrifuged before applying it to the column. The most convenient and reproducible sample injection is via the Amersham Biosciences valves V-7, PV-7, MV-7 or PMV-7. Loading capacity is 0.5-1 ml depending on the sample and the flow rate.

Sample elution

The flow rate for elution should not exceed 6 ml/min. For best results use between 2-6 ml/min, the lower flow rate when the sample size exceeds 0.5 ml. The column is designed for samples of 0.5 ml which normally elute in a peak volume of 1-2 ml at a flow rate of 6 ml/min.

Automation

Repeated injections in two-dimensional chromatography, e.g. comprising gel filtration on a Fast Desalting Column and ion exchange on MonoBeads[®] may be automated with FPLC System consisting of two pumps for gradient formation, one P-1 pump if you wish to have automated sample loading, a Liquid Chromatography Controller, (LCC-500, LCC-500 Plus or LCC-501 Plus), two injection valves, e.g. Motor Valves MV-7, one selection valve, e.g. Motor Valve MV-8, one ValvePSV-100, and one Superloop. A practical example of a set-up is illustrated in Figure 1. The methods programmed may be similar to the ones shown in Figure 2. To program your own methods, refer to the manual for LCC-500, LCC-500 Plus or LCC-501 Plus controller.

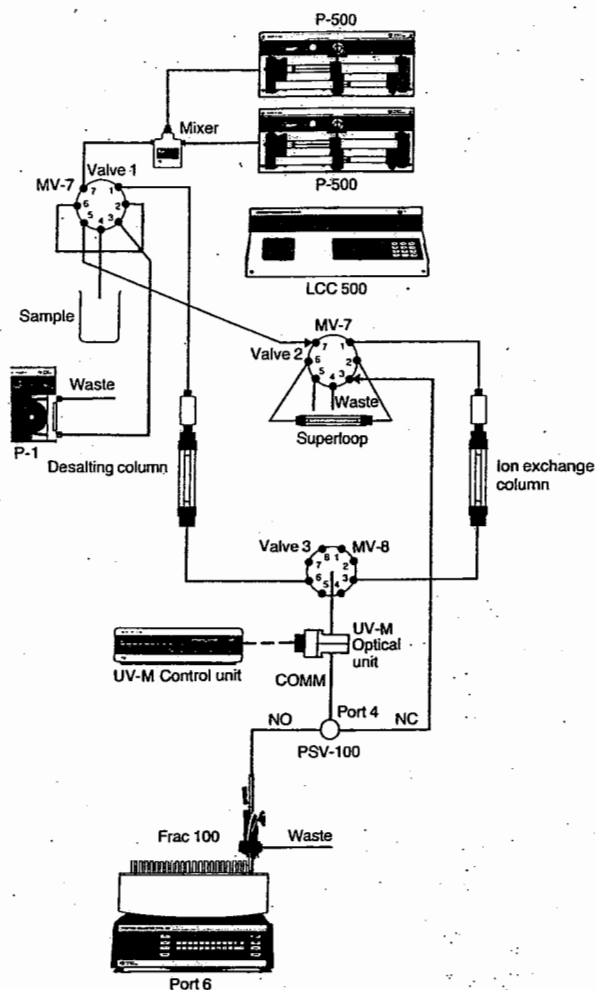


Fig. 1. A schematic view of a system for automated desalting.

Increased back-pressure and column cleaning

Change the top filter and clean the column if you observe

- an increased back-pressure
- a gap between adaptor and filter. (The adaptor can be adjusted to the gel bed by turning the red ring clockwise, but the gap is an indication that the filter or the gel top is dirty.)
- a loss of resolution

Perform the following steps in sequence until the performance is restored.

1. Change the top filter before cleaning since this filter is loose and may (if blocked) compress the gel. Follow the instructions supplied with the Filter Kit (see "Spare parts and accessories").
2. Clean the column with 0.1 M NaOH. Use a flow rate that gives a back-pressure well below the pressure limit (1 MPa, 10 bar, 140 psi).
3. If necessary repeat step 2 with reversed flow. Make sure the top adaptor is adjusted to the gel bed.

**Start-up method,
buffer exchange**

METHOD NO. 0		
0.0	VALVE.POS	1.3
0.0	VALVE.POS	2.3
0.0	VALVE.POS	3.6
0.0	CONC %B	0.0
0.0	ML/MIN	1.00
0.2	WASH A.B	1.1
0.2	VALVE.POS	1.1
0.2	ML/MIN	4.00
25.0	CONC %B	0.0
25.0	ML/MIN	1.50
25.0	VALVE.POS	1.3
25.0	VALVE.POS	2.1
25.0	VALVE.POS	3.3
30.0	CONC %B	0.0
30.0	CONC %B	100
40.0	CONC %B	100
40.0	CONC %B	0.0
45.0	CONC %B	0.0

Method for ion exchange

METHOD NO. 3		
0.0	VALVE.POS	1.3
0.0	VALVE.POS	3.3
0.0	CONC %B	0.0
0.0	ML/MIN	2.00
1.0	CONC %B	0.0
1.0	CM/ML	0.50
1.0	PORT.SET	6.1
1.0	VALVE.POS	2.2
1.0	CLEAR DATA	
1.0	MONITOR	1
1.0	LEVEL %	5.0
1.0	ML/MARK	2.0
1.0	INTEGRATE	1
6.0	CONC %B	0.0
6.0	VALVE.POS	2.1
31.0	CONC %B	50.0
35.0	CONC %B	100
40.0	CONC %B	100
40.0	CONC %B	0.0
40.0	PORT.SET	6.0
40.0	INTERGRATE	0
40.0	PRT PK	1.10
46.0	CONC %B	0.0
46.0	CALL METH	7

**Control method, desalting
and ion exchange**

METHOD NO. 1		
0.0	ML/MIN	0.10
0.0	LOOP TMS	1
0.0	CALL METH	2
0.0	CALL METH	3
0.0	END OF LOOP	

Method for fast desalting

METHOD NO. 2		
0.0	CONC %B	0.0
0.0	VALVE.POS	1.1
0.0	VALVE.POS	2.1
0.0	VALVE.POS	3.6
0.0	ML/MIN	1.00
0.1	ML/MIN	4.00
0.1	CALL METH	4
0.1	CM/ML	0.50
0.1	CLEAR DATA	
0.1	MONITOR	1
0.1	LEVEL %	5.0
0.1	ML/MARK	1.0
0.1	INTEGRATE	1
0.1	PS CALL	5
0.1	PE CALL	6
5.0	INTEGRATE	0
5.0	PRT PK	1.05
6.0	CONC %B	0.0
6.0	CM/ML	0.00

Sample loading

METHOD NO. 4		
0.0	VALVE.POS	1.1
0.0	FLOW C	2.00
2.0	FLOW C	0.00
2.0	VALVE.POS	1.2

Peak collection

METHOD NO. 5		
0.0	PS CALL	0
0.0	PORT.SET	4.1

METHOD NO. 6		
0.0	PE CALL	0
0.2	PORT.SET	4.0

**Method for equilibration,
Fast Desating Column**

METHOD NO. 7		
0.0	CONC %B	0.0
0.0	ML/MIN	1.00
0.0	VALVE.POS	1.1
0.0	VALVE.POS	3.6
0.0	CM/ML	0.50
0.1	ML/MIN	4.00
13.0	VALV.POS	2.2
13.1	PORT.SET	4.1
15.0	PORT.SET	4.0
15.1	VALVE.POS	2.1
16.0	CONC %B	0.0

Fig. 2. Methods programmed on LCC-501 Plus for automated two-dimensional chromatography with FPLC® System.

Repacking the column

You may try to restore a gel bed which has been damaged, but not over-compressed, by repacking. You need the Packing Equipment HR 10 and a pump which gives an accurate flow at medium pressures, e.g. Pump P-500. Pack the gel slurry in 0.1 M NaCl at a flow rate of 6 ml/min. When the gel has settled, remove the packing reservoir, insert the adaptor, and continue to pack for four column volumes at 6 ml/min.

Note: We can not guarantee that a repacked column will exhibit the same high performance as a new one.

Storage and prevention or microbial growth

Store the column in water or buffer containing 0.02% NaN₃ (sodium azide). Avoid prolonged exposures to extremes of pH. You may also store in 20% ethanol, but be careful; make sure the gel is clean and use low flow rates when adding or removing the ethanol solution.

Further information

For further information on columns and equipment for FPLC, please contact your local representative of Amersham Biosciences.

Spare parts and accessories

The part number refers to the number in the exploded view of the column (Fig 3.). When ordering, please quote according to the designations and code numbers listed below.

Part No.	Designation	Code No.	No. per pack
1-4, 9	Top assembly HR 10	18-1541-01	1
6-7, 9	Bottom assembly HR 10	18-1542-01	1
5	Protective jacket HR 10/10	19-7449-01	1
8	Chromatographic tube HR 10/10	19-7464-01	1
	Filter kit HR 10	18-3575-01	10
	Filter tool	18-3590-01	1
	Flanging/Start-Up kit 120 V	19-5079-01	1
	220 V	19-5090-01	1
9	Tubing connectors* Capillary tubing, o.d. 1.8 mm, i.d. 0.5 mm, L 2 m	19-7477-01	1
	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	18-1023-85	1
	Union (1.8 mm to 1/16") (Waters™ compatible)	18-3405-01	2
	(Swagelok™ compatible)	18-3406-01	2
	Union, M6 female/1/16" female (Valco™ compatible, titanium)	18-3859-01	1
	Union, M6 female/1/16" male (Valco™ compatible, plastic)	18-3858-01	1
	Union, M6 female/ M6 female (titanium)	18-3856-01	1
	SRTC-2 (M6 female/ M6 female, plastic)	19-2143-01	5
	Prefilter	19-5084-01	1
	Filters + O-rings (prefilter)	19-5082-01	5 + 2
	Packing Equipment HR 10	18-1443-01	1

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

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

Fig. 3.

