Mono S HR 16/10

Instructions for Use

Columns prepacked with Mono S™ are designed for fast, high resolution cation exchange separations of proteins, peptides, polynucleotides and other biomolecules.

Introduction

Mono S HR 16/10 (20 mL) cation exchange columns can be used with ÄKTAdesign systems and other high performance chromatographic systems. These instructions will help you obtain the best results from your column.

Other columns available in the series of MonoBeads™ are Mono Q™ (anion exchange) and Mono P™ (chromatofocusing).

Unpacking

Please check the delivery against the packing list.

Designation	No. supplied	Code No.
Mono S HR 16/10	1	17050701
Filter kit HR 16	1 (10 filters)	18358501
Filter tool	1	18115320
Wrench	1	19748101
Instructions	1	

Quality control test

To guarantee the quality of Mono S HR 16/10 each column is efficiency tested. Each media batch undergoes a function test to ensure reproducible results.

Connecting the column to ÄKTAdesign system

Step	Action
1	The 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 your column.
2	Connect the shorter preflanged tubing (the outlet) via a union which adapt the M6 connector to 1/6" tubing (see *Spare parts and accessories, on page 5*) to the detector.
3	Connect the longer preflanged tubing (the inlet) via a union which adapt the M6 connector to 1/16" tubing (see *Spare parts and accessories, on page 5*) to a valve which can be positioned for samle injection and elution. 4.
4	It is recommended to use a prefilter with the prepacked column (see *Spare parts and accessories, on page 5*).

Columns prepacked with Mono S can be used with any HPLC system if the pump can provide precise and accurate flow at relatively low back-pressures.

Important before use

The glass columns HR 16/10 are stable up to 3 MPa (30 bar, 450 psi). Set the pressure limit control accordingly.

The gel is delivered in a 20% ethanol-water solution with sodium as the counter-ion, and should be equilibrated according to the following steps.

Step	Action
1	Wash away the packing solution with 100 mL of start buffer (low ionic strength).
2	Change to the desired counter-ion by washing with 200 mL of eluent B (high ionic strength).
3	Equilibrate with 100 mL of starting buffer. Before applying the sample, make sure that the ion exchange bed has reached equilibrium.

To ensure long column life, always filter eluents and centrifuge or filter samples before applying them to the column.

Flow rates up to 10 mL/min are recommended depending on requirements and eluent viscosity.

Media properties

Mono S is a strong cation exchanger based on a beaded hydrophilic resin with one of the narrowest particle size distributions available. The chemistry of the beads was developed at Cytiva and the monodispersity was accomplished through a unique process developed by Prof. John Ugelstad of SINTEF, Trondheim, Norway.

Mono S has a particle size of 10 µm. The absence of fines gives the packed columns large void volumes (40%) and therefore low back-pressures.

The charged group on the gel is –CH2–SO3 –. Ionic capacity of the gel is 0.14 to 0.18 mmol/mL. Separations of substances with molecular weights up to 107 have been carried out successfully. Protein capacity is normally in the range 20 to 50 mg/mL gel.

The amount of non-specific adsorption to MonoBeads is negligible. Recovery of enzyme activity is normally greater than 80%.

Chemical and physical stability

Mono S HR 16/10 columns can be used in aqueous media in the pH range 2 to 12 and for cleaning in the pH range 2 to 14.

Aqueous solutions of urea, ethylene glycol and similar compounds may also be used.

Non-ionic or anionic detergents can be used but be sure to equilibrate the media with the detergent solution beforehand. Cationic detergents should not be used with Mono S.

Mono S HR 16/10 columns are stable in alcohol/water solutions (C1-C4 alcohols). Dimethyl sulphoxide, dimethyl formamide, formic acid and similar solvents change the separation properties of the media so we do not recommend them. All oxidizing and other reactive substances should be avoided.

The glass columns HR 16/10 are stable up to 3 MPa. Using an aqueous solution at a flow rate of 8.0 mL/min, the operating pressure is generally less than 2 MPa.

Columns may be operated at temperatures between 4°C and 40°C.

Choice of elution conditions

Cation exchangers should be used with anionic or zwitterionic buffers, e.g. MES (2-[N-Morpholino] ethane sulphonic acid). Avoid cationic buffers since they bind to Mono S. Buffer concentrations should be at least 10 mM. Cationic detergents bind to Mono S and should not be used. Anionic (e.g. SDS) or non-ionic detergents (e.g. octylglucoside) may be used.

The table below gives recommended buffers for various pH intervals. This information has been determined with data from numerous runs peformed in our application laboratories. All values were determined at room temperature.

Sodium is the most common cationic counterion. A concentration of 1 M (e.g. NaCl) in buffer B is recommended to give a cleaning effect at 100%.

Twenty millitres/mL gel is a reasonable gradient volume. If the protein of interest is not eluted, then increase the gradient volume and the cation concentration at the end of the separation.

Table 1. Recommended buffers for vaious pH intervals

pH interval	Buffer[1]	Concentration[2]	Anion	pKa (25°C)	dpKa[3] dT (°C)
3.8 to 4.3	formate	50 mM	Na^+, Li^+	3.75	+0.0002
4.3 to 4.8	succinate butanedioic acid	50 mM	Na^+	4.20	–0.0018
4.8 to 5.2	acetate	50 mM	Na^+, Li^+	4.76	+0.0002
5.0 to 6.0	malonate	50 mM	Na^+, Li^+	5.69	
5.5 to 6.7	MES	50 mM	Na^+, Li^+	6.15	–0.0110
6.7 to 7.6	phosphate	50 mM	Na^+	7.20	–0.0028
7.6 to 8.2	HEPES	50 mM	Na^+, Li^+	7.55	–0.0140
8.2 to 8.7	BICINE	50 mM	Na^+	8.35	–0.0180

[1] Buffers are made up with the acid form of the anions.

[2] Buffer concentration gradients may improve resolution.

[3] When working at different temperatures, allow for changes in the pKa.

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 to 0.45 µm sterile filter. Either centrifuge (10 000 × g 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.

The samples should be fat-free. Turbid solutions can decrease the column lifetime.

When possible, dissolve the sample in start buffer. The buffer is easily exchanged by gel filtration with Sephadex™ G-25 , such as in prepacked HiPrep 26/10 Desalting column (15 mL sample volume). For smaller sample volumes, HiTrap Desalting columns (1.5 mL) or PD- 10 columns (2.5 mL) may be used.

Note: *Careful handling of solutions and samples increases the life time of the column considerably.*

Column equilibration

To equilibrate the column for first-time use or for changing counter-ions, proceed according to steps 1 to 3 below.

Step	Action
1	Wash with 100 mL of start buffer (low ionic strength).
2	Change to the desired counter-ion by washing with 200 mL of eluent B (high ionic strength).
3	Equilibrate with 100 mL of the start buffer. Before applying a sample, equilibrate with start buffer until the base-line is stable

Note: *Be sure to equilibrate completely if using detergents.*

Sample application

Make sure the sample is recently filtered or centrifuged before applying it to the column (see *Eluent and sample preparation, on page 2*). Protein loading capacity is generally 20 to 50 mg/mL media, or 100 mg per single peak, depending on the sample. This loading usually gives good resolution but the capacity varies for different proteins.

Sample elution

Flow rates can be varied with little effect on resolution. Up to 10 mL/min is recommended. A gradient volume of 20 mL/mL media is generally sufficient. Larger gradient volumes generally improve resolution but also increase peak dilution.

Column re-equilibration

To re-equilibrate the column, inject 10 mL of a 1 M solution of the elution salt and equilibrate with the start buffer. If changing counterions, follow the column equilibration procedure mentioned previously.

Method optimisation and scaling up

Sample elution is carried out by applying a concentration gradient to the column. Optimal flow rate and gradient shape depend on the separation problem and the cation used. To save sample and buffer salts, it is recommended to optimise the separation on Mono S 5/50 GL. The separation can then be directly scaled up to Mono S HR 16/10. Below are some general recommendations:

1. Best separating pH and buffer system for the sample can be determined by scouting on Mono S 5/50 GL. High flow rates (up to 2 mL/min) and small gradient volumes (20 mL) may be used to save time. The elution ionic strength for each separated component is independentof the flow rate (*Fig. 1, on page 3*).

2. Optimise the gradient volume to get the best resolution. The resolution increases with increasing gradient volume (*Fig. 2, on page 3*). At the same time, the elution ionic strength for the separated components is reduced (*Fig. 3, on page 3*).
3. If necessary, resolution can be increased by reducing the flow rate (*Fig. 4, on page 3*).
4. Determine the maximum loading that still provides acceptable resolution by increasing the sample amount.
5. Scale up the separation to Mono HR 16/10 as below:

	HR 16/10
Sample amount	× 20
Gradient volume	× 20
Flow rate	× 10 or up to 10 mL/min

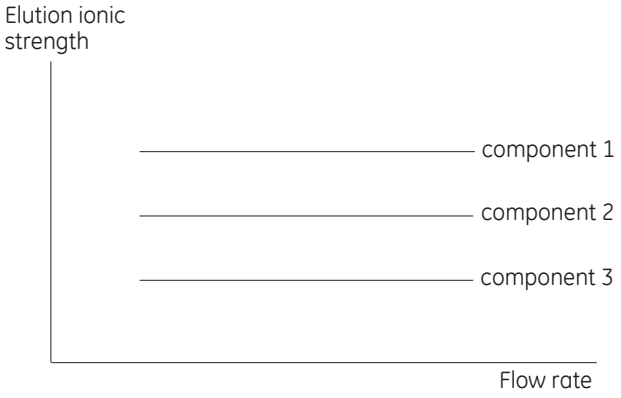


Fig 1.

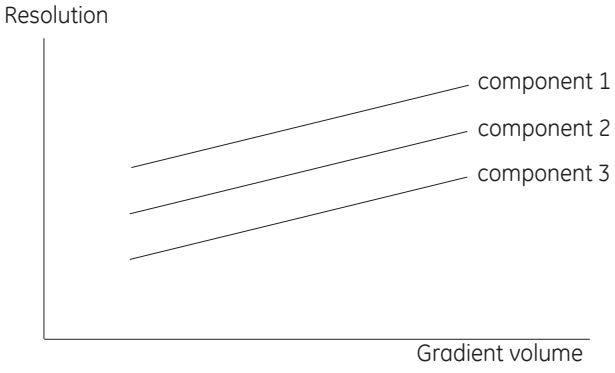


Fig 2.

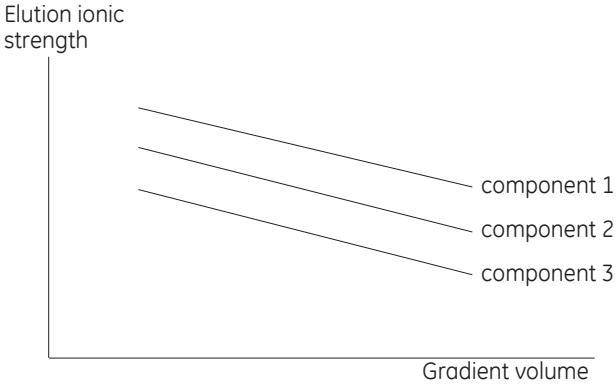


Fig 3.

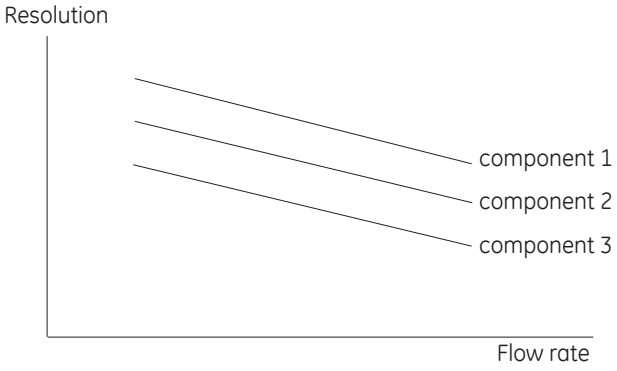


Fig 4.

Increased back-pressure

If increased back-pressure becomes a problem, perform the following steps in sequence until normal pressures are obtained (a good routine is always to record the backpressure in your running protocols, e.g. just prior to sample injection).

Step	Action
1	Check the flanges of the tubings and reflange or exchange assemblies if damaged.
2	Turn the red adjusting ring on the top adaptor half a turn counter-clockwise. (The adaptor should still be close to the gel bed, without pressing against it, otherwise the back-pressure will be increased). Reverse the flow direction and pump 40 mL of buffer at 5 mL/ min. Return to normal flow direction and run for 4 min at 10 mL/min. Readjust the top adaptor.
3	Check the top filter (Filter kit HR 16) and change if contaminated (see *Spare parts and accessories, on page 5*).
4	If the problem persists, clean the column according to the procedure described under *Column cleaning, on page 3*.

Column cleaning

The following observations indicate that column washing may be necessary.

- increased back-pressure – please check the filters first
- colour change at the top of the column
- loss of resolution
- decreased samle recoveries

It is best to avoid these problems by washing the column routinely (e.g. every fifth or tenth run) following steps 1 to 8 below. More frequent washing may be necessary if complex samples are applied. Steps 1 to 10 are guidelines. Use your knowledge about possible contamination to evaluate which cleaning method is suitable.

Step	Action
1	Connect the column inlet to the detector. Set the sensitivity to 0.2 AUFS.

Step	Action
2	Make sure there is no space between the media and adaptor. Start a reversed flow at a rate of 2 to 5 mL/min. Carry our steps 3 to 8 in sequence, ensuring each time that the monitored peaks are identical in size before proceeding to the next step. Rinse with water or buffer (A) after step 3 to 7.
3	Inject 5 mL 2 M NaCl solution.
4	Inject 5 mL 2 M NaOH.
5	Inject 5 mL 2 M NaCl solution.
6	Inject 5 mL 1 M HCl solution.
7	Inject 10 mL 75% acetic acid or 1% TFA.
8	Inject 5 mL 2 M NaCl solution or a 2 M solution which has the same counter-ion as the solution used for elution.
9	If the column performance is still not restored, try leaving the column overnight in a solution of 1 mg/ mL pepsin, 0.1 M acetic acid and 0.5 M NaCl. (Instead of pepsin, you may try other enzymes, e.g. DNase, depending on the contamination.) For enzymatic washing, the solution should be at a temperature of 37°C. After the enzymatic cleaning, perform the chemical scrubbing (steps 3 to 8) again.
10	As a last attempt to restore performance, suspend 2 to 3 mm of the media top and remove it with a Pasteur pipette. Adjust the adaptor to eliminate the space above the media bed.

More information on column cleaning is found in *Ion Exchange Chromatography & Chromatofocusing, Principles and Methods*.

Checking the column packing

A well packed column is essential for high performance chromatography even though high efficiency (that is, number of plates/column) is less critical in gradient techniques than isocratic techniques. For best performance, make sure there is no space between the top adaptor and the gel bed (adjust the adaptor by clockwise rotation of the red adjusting ring on the top of the column), and that the column is clean (see *Column cleaning, on page 3*).

If you suspect column packing to the cause of reduced resolution, run a sample and note the shape of the sample zone. Cytochrome c (Sigma type 3) is a good test protein, since it is coloured and readily available.

Step	Action
1	Dissolve the cytochrome c, 1 mg/mL in 10 mM phosphate buffer, pH 7.0.
2	Apply approximately 1 mL of the cytochrome c solution to the column.
3	Elute with 10 mM phosphate buffer, containing 1 M NaCl.

Note the shape of the protein zone. When injected it should be a narrow, horizontal band at the top of the column. When eluted with 100% eluting buffer, the zone should move down the column as a band. If the band is wavy, diffuse or not horizontal during elution, adjust the top adaptor and, if not already done, clean the column. column. When eluted with 100% elution buffer, the zone should move down the column as a band. If the band is wavy, diffuse or not horizontal during elution, adjust the top adaptor and, if not already done, clean the column.

Effiency 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 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
W_h = peak width at half peak height
H^{-1} = number of theoretical plates/m

Sample:	Triglycerine, 0.1 mg/mL, 500 µL
Eluent:	0.01 M H_2SO_4, pH 2.0 (KOH)
Flow rate:	5.0 mL/min

Function test

An alternative to the efficiency test is the function test described here.

Mono S HR 16/10

Experimental:	
Sample:	500 µL solution containing 1. Wheat germ lectin, 3 mg/mL 2. β-lactoglobulin, 1.5 mg/mL
Buffer A:	0.02 M Formic acid, pH 4.0 (NaOH)
Buffer B:	0.02 M Formic acid with 0.75 M LiCl, pH 4.0 (NaOH)
Flow rate:	10 mL/min
Gradient:	0.5–100% B in 20 min
Detector:	UV-M/UV-MII, 280 nm, 0.2 AUFS
Chart speed:	0.5 cm/min

Storage and prevention of microbial growth

Before storing for long periods, wash the column sequentially with 200 mL of 0.5 M NaCl, 100 mL of water and 100 mL of 20% ethanol. The column should be stored in 4°C to 30°C.

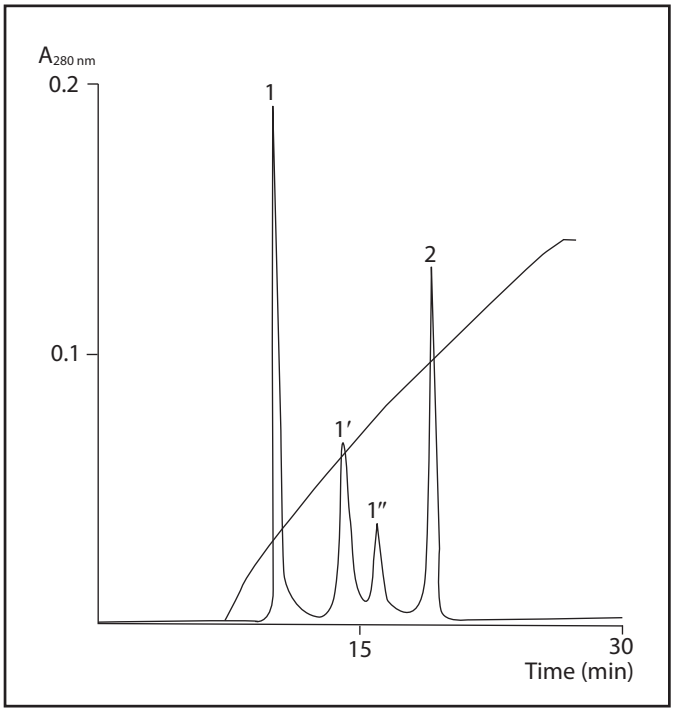
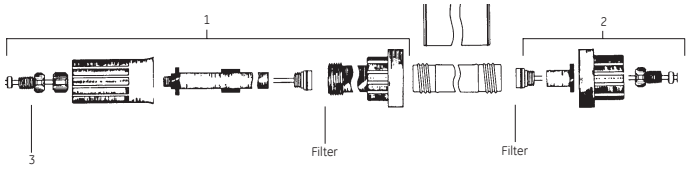


Fig 5. Typical chromatogram from a function test of Mono S HR 16/10.



Spare parts and accessories

Pos.	Designation		No. per pack	Code No.
1	Top assembly	HR 16	1	18154401
2	Bottom assembly	HR 16	1	18154501
	Filter kit	HR 16	10	18358501
	Filter tool			18115320
3	Tubing connectors[1]		5	19747601
	Capillary tubing (o.d. 1.8 mm, i.d. 0.5 mm)		2 m	19747701
	Prefilter		1	19508401
	Filters + O-rings (prefilter)		5 + 2	19508201
	Flanging/Start-Up kit		1	19507901
	120V		1	19509001
	220V			
	Sample loops 1 mL, 2 mL		1 of each	18589701
	Superloop 10 mL		1	19758501
	Superloop 50 mL		1	19785001
	Solvent resistant O-ring (for the Superloop)		1	18630001
	Union, M6 female/1/16″ female, stainless steel (Waters[2] compatible)		1	18340501
	Union, M6 female/1/16″ female, titanium (Valco[2] compatible)		1	18385901
	Union, M6 female/1/16″ male, plastic (Valco compatible)		1	18385801
	PD 10		30	17085101
	HiPrep 26/10 Desalting		1 × 53 mL	17508701
	HiTrap Desalting		5 × 5 mL	17140801

[1] You need the Flanging/Start-Up kit to attach new tubing connectors.

[2] Waters is our abbreviation for the fittings produced by Millipore Corp. Swagelok is a registered trademark of the Crawford Fitting Company. Valco is a trademark of Valco Instrument Co. Inc.

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