Mono S HR 5/5

Columns prepacked with Mono S™ are designed for fast, high performance cation exchange separations of biomolecules.

Mono S HR 5/5 (1 ml) has been designed for operation with FPLC™ System, but can also be used with HPLC systems.

Unpacking

Please check the delivery against this list

Designation	Code No	No. supplied
Mono S HR 5/5	17-0547-01	1
Wrench	19-7481-01	1
Instructions		1

Quality control tests

To guarantee Mono S HR 5/5 is a product of high quality the efficiency of each column is tested. Each media batch undergoes a function test to ensure reproducible results.

Connecting the column to FPLC System

1. Connect the shorter preflanged tubing (the outlet) to the detector.
2. Connect the longer preflanged tubing (the inlet) to a valve for sample injection and elution e.g. Amersham Biosciences valve V-7 or PV-7 or motor valve MV-7 or PMV-7. When using the PV-7 or the PMV-7 valves, connect a Union, M6 female/1/16" male (Code No. 18-3858-01), between the flanged tubing and the valve.
3. We recommend you to use a prefilter to protect the column (see "Spare parts and accessories").

Connecting the column to HPLC systems

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. The column should be connected as described for FPLC System via two unions which adapt the M6 connector to 1 /16" tubing (see "Spare parts and accessories").

Important before use

The glass column HR 5/5 is stable up to 10 MPa (100 bar, 1 500 psi). To avoid altering the column packing, we recommend a maximum back-pressure of 5 MPa (50 bar, 750 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.

1. Wash away the packing solution with 5 ml of start buffer (low ionic strength).
2. Change to the desired counter-ion by washing with 10 ml of eluent B (high ionic strength).
3. Equilibrate with 5 ml of the start buffer before applying the sample, or until stable baseline.

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

We recommend a flow rate of 0.5–2 ml/min depending on requirements and eluent viscosity.

Gel 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 Amersham Biosciences 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 $-CH_2-SO_3^-$.
Ionic capacity of the gel is 0.14–0.18 mmol/column.
Separations of substances with molecular weights up to 10^7 have been carried out successfully. Protein capacity is normally in the range 20–50 mg/column.

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

Amersham Biosciences

Chemical and physical stability

Mono S HR 5/5 can be used in aqueous media in the pH range 2–12. Aqueous solutions of urea, ethylene glycol and similar compounds may also be used. For short term use, for example during cleaning pH stability is 2–14.

Non-ionic or anionic detergents can be used but be sure to equilibrate the gel with detergent solution beforehand.

Cationic detergents should not be used with Mono S.

Mono S HR 5/5 columns are stable in alcohol/water solutions (C_1–C_4 alcohols). Dimethyl sulphoxide, dimethyl formamide, formic acid and similar solvents change the separation properties of the gel, so we do not recommend them. All oxidizing and other reactive substances should be avoided.

The glass column HR 5/5 is stable up to 10 MPa. We recommend an operating pressure of up to 5 MPa for best performance. Using an aqueous solution at a flow rate of 1.0 ml/min, Mono S HR 5/5 generally operates at a back-pressure of 1–1.5 MPa.

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

Choice of elution conditions

Cationic exchangers should be used with anionic or zwitterionic buffers e.g. MES (2-(N-Morpholino) ethane sulphonic acid). Avoid cationic buffers and detergents since they bind to Mono S. Anionic (e.g. SDS) or non-ionic detergents (e.g. octylglucoside) may be used. Buffer concentrations should be at least 10 mM.

Table 1 gives recommended buffers for various pH intervals. This information has been determined with data from numerous runs performed in our application laboratories. All values were determined at room temperature.

Sodium is the most common cationic counter-ion. We recommend a concentration of 1 M (e.g. NaCl) in buffer B to give a cleaning effect at 100%.

Twenty millilitres 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.

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 (10 000 × *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.

Note: 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. For smaller volumes, you may use the Fast Desalting Column HR 10/10 (1 ml) or PD-10 columns (2.5 ml).

Note: Careful handling of solutions and samples increases the lifetime of the column considerably.

Column equilibration

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

1. Wash with 5 ml of start buffer (low ionic strength).
2. Change to the desired counter-ion by washing with 10 ml of eluent B (high ionic strength).
3. Equilibrate with 5 ml of the start buffer.

Before applying a sample, equilibrate with start buffer until the baseline is stable.

Note: Be sure to equilibrate completely if using detergents.

Table 1. Recommended buffers and cations for various pH intervals.

pH interval	Buffer	Concentration[1]	Cation	pKa (25°C)	dpKa[2] / dT (°C)
3.8–4.3	formate[3]	50 mM	Na^+, Li^+	3.75	+0.0002
4.3–4.8	succinate (butanedioic acid)	50 mM	Na^+	4.20	–0.0018
4.8–5.2	acetate	50 mM	Na^+, Li^+	4.75	+0.0002
5.0–6.0	malonate	50 mM	Na^+, Li^+	5.69	
5.5–6.7	MES	50 mM	Na^+, Li^+	6.10	–0.0110
6.7–7.6	phosphate	50 mM	Na^+	7.20	–0.0028
7.6–8.2	HEPES	50 mM	Na^+, Li^+	7.48	–0.0140
8.2–8.7	BICINE	50 mM	Na^+	8.26	–0.0180

1 Buffer concentration gradients may improve resolution.
2 When working at different temperatures, allow for changes in the pKa
3 Buffers are made up with the acid form of these anions

Sample application

Make sure the sample is recently filtered or centrifuged before applying it to the column. The most convenient and reproducible sample injection is via the valves V-7, MV-7, PV-7 or PMV-7. Large volumes (up to 50 ml) can be applied from a Superloop (see "Spare parts and accessories"). Protein loading is generally 20–50 mg/column, or approx. 5 mg per single peak, depending on the sample. This loading usually gives good resolution but the capacity varies for different proteins.

Sample elution

We recommend flow rates up to 2.0 ml/min. Flow rates can be varied with little effect on resolution. A gradient volume of 20 ml is generally sufficient. Larger gradient volumes generally improve resolution but also increase peak dilution.

Column re-equilibration

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

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 back-pressure in your running protocols, e.g. just prior to sample injection).

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 2 ml of buffer at 0.5 ml/min. Return to normal flow direction and run for 2 min at 1 ml/min. Readjust the top adaptor.

3. Check the top filter (Filter HR 5) and change if contaminated (see "Spare parts and accessories").

4. If the problem persists, clean the column according to the procedure described under "Column cleaning" below.

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 sample recoveries

It is best to avoid these problems by washing the column routinely (e.g. every fifth or tenth run) following steps 1–9 below. More frequent washing may be necessary if complex samples are applied.

Steps 1–11 are guidelines. Use your knowledge about possible contamination when choosing a cleaning method.

The following is a list of recommended washing solutions. Always ensure the baseline is stable before proceeding to the next wash.

- 2 M NaCl
- 2 M NaOH
- 1 M HCl
- 75% acetic acid
- 1% TFA
- 0.5% SDS in 0.5 M NaOH
- 30% acetonitrile
- 0.5% non-ionic detergent (e.g. octylglucoside) in 3 M HAc

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

2. Make sure there is no space between the gel and adaptor. Start reversed flow at a rate of 0.25–0.50 ml/min.

3. Inject 500 µl 2 M NaCl solution. Rinse with 5–10 ml of water or buffer (A).

4. Inject 500 µl of 0.5% SDS in 0.5 M NaOH. Rinse with 5–10 ml of water or buffer (A). (To be performed at room temp. as SDS can crystallise at lower temperatures.)

5. Inject 500 µl 2 M NaCl solution. Rinse with 5–10 ml of water or buffer (A).

6. Inject 500 µl 2 M NaOH. Rinse with 5–10 ml of water or buffer (A).

7. Inject 500 µl 1 M HCl. Rinse with 5–10 ml of water or buffer (A).

8. Inject 500 µl 75% acetic acid or 1% TFA. Rinse with 5–10 ml of water or buffer (A).

9. Inject 1 ml of 2 M NaCl solution or a 2 M solution which has the same counter-ion as the solution used for elution. Rinse with 5–10 ml water or buffer and equilibrate.

10. 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 3–9) again.

11. As a last attempt to restore performance, suspend 2–3 mm of the gel top and remove it with a pasteur-pipette. Adjust the adaptor to eliminate the space above the gel bed.

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 not as critical in gradient techniques as in 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 ensure that the column is clean (see section "Column cleaning").

If you suspect column packing to be 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.

1. Dissolve the Cytochrome C, 1 mg/ml in 10 mM phosphate buffer pH 7.0.
2. Apply approximately 100 µl 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% 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.

Efficiency and function tests

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

Experimental

Sample: 20 µl of triglycine, 0.1 mg/ml
Eluent: 0.01 M H_2SO_4, pH 2.0 (KOH)
Flow rate: 0.5 ml/min
Detector: UV-M, 214 nm, 0.05 AUFS
Chart speed: 3 cm/min

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

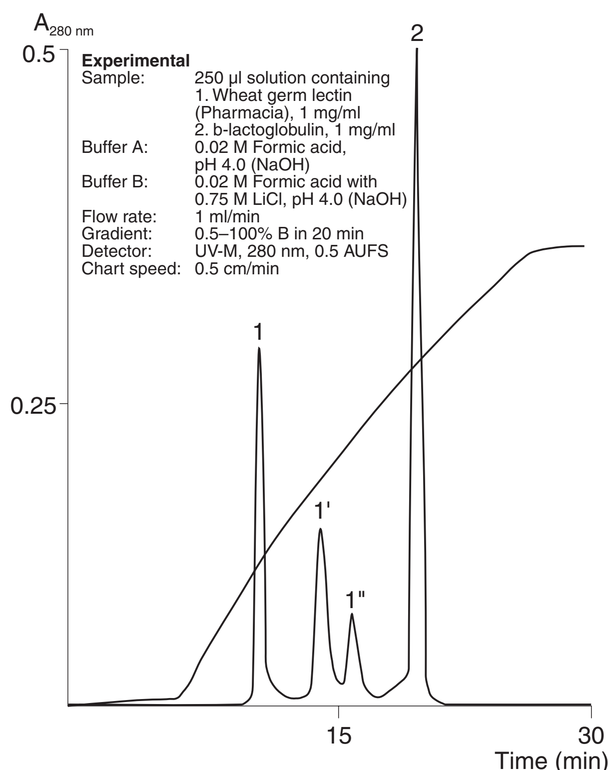

Fig 1. Typical chromatogram from a function test of Mono S HR 5/5.

Storage and prevention of microbial growth

Before storing for long periods, wash the column sequentially with 5 ml of 0.5 M NaCl, 5 ml of water and 5 ml of 20% ethanol. The column should be stored between 4 and 30 °C.

Scaling up

For scaling up there are two column sizes available:

Column	Bed volume	Code No.
Mono S HR 10/10	8 ml	17-0557-01
Mono S HR 16/10	20 ml	17-0507-01

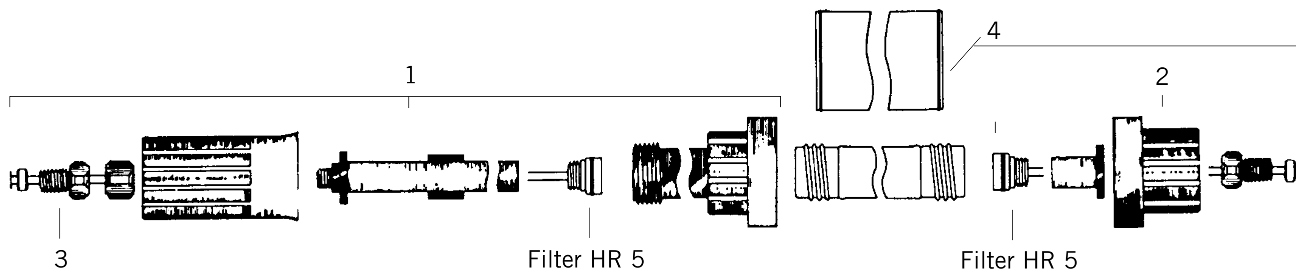

Fig 2.

Methods successfully developed on Mono S HR 5/5 can be directly scaled up to Mono S HR 10/10 and HR 16/10 using simple scale factors:

Sample loading	×	increase in gel volume	(8 or 20 times respectively)
Gradient volume	×	increase in gel volume	(8 or 20 times respectively)
Flow rate	×	increase in column area	(4 or 10 times respectively)

For more information on scaling up, contact your local Amersham Biosciences representative.

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

Spare parts and accessories

Pos.	Designation	Code No.	No. per pack
1	Top assembly HR 5	18-6352-01	1
	Filter HR 5	18-6353-01	10
2	Bottom assembly HR 5	18-6351-01	1
3	Tubing connectors*	19-7476-01	5
4	Protective jacket HR 5/5	19-7447-01	1
	Capillary tubing (o.d. 1.8 mm, i.d. 0.5 mm)	19-7477-01	2m
	Prefilter	19-5084-01	1
	Filters + O-rings (prefilter)	19-5082-01	5+2
	Flanging/Start-Up kit 120V	19-5079-01	1
	220V	19-5090-01	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	18-1023-83	
	Solvent resistant O-ring (for the Superloop)	18-6300-01	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
	Domned nut, M6	18-2450-01	4
	PD 10	17-0851-01	30

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

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