Mono Q HR 5/5

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

Introduction

Mono Q HR 5/5 (1 ml) is an anion exchange column which has been designed for operation with FPLC™ System. These instructions is a guide for obtaining the best results from the column.

Unpacking

Please check the delivery against this list.

Designation	Code No	No. supplied
Mono Q HR 5/5	17-0546-01	1
Wrench	19-7481-01	1
Instructions		1

Quality control tests

To guarantee the quality of Mono Q HR 5/5, each column is efficiency 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 which is used for sample injection and elution e.g. 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.
3. It is recommended to use a prefilter with the prepacked column (see “Spare parts and accessories”).

Connecting the column to HPLC systems

Columns packed with Mono Q 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, 1500 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 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. Wash with 10 ml of eluent B (high ionic strength).
3. Equilibrate with 5 ml of the start buffer. Before applying the sample, equilibrate with start buffer until stable baseline.

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

Recommended flow rates are 0.5–2 ml/min depending on requirements and eluent viscosity.

Gel properties

Mono Q is a strong anion 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 Q beads have 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 $-CH_2-N^+(CH_3)_3$. Ionic capacity of the gel is 0.27–0.37 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. Enzyme activity recoveries are normally greater than 80%.

Chemical and physical stability

Mono Q 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 in a cleaning procedure, the pH stability is 2–14.

Non-ionic or cationic detergents can be used but be sure to equilibrate the gel with the detergent solution beforehand. Anionic detergents should not be used with Mono Q.

Mono Q HR 5/5 is 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 Q HR 5/5 generally operates at a back-pressure of 1–1.5 MPa.

Choice of elution conditions

Anion exchangers should be used with cationic or zwitterionic buffers, e.g. histidine. Avoid anionic buffers since they bind to Mono Q. Buffer concentrations should be at least 10 mM. Anionic detergents, such as SDS, bind to Mono Q and should not be used. Cationic or non-ionic detergents (e.g. octylglucoside) may be used.

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.

Specific anions have different elution strengths. Table 2 gives various anions and their recommended concentrations at the end of the separation and to make up buffer B to give it a cleaning effect at 100%.

Twenty millitres is a reasonable gradient volume for all the anions in Table 2. If the protein of interest is not eluted, then increase the gradient volume and the anion concentration at the end of the separation.

Table 2. Anions and recommended concentrations

Anion	Concentration (M) End of separation	Buffer B
SO^{2-}	0.15	0.50
Cl^-	0.35	1.00
$HCOO^-$	0.60	1.70
OA_{c-}	0.70	2.00

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 x *g* for 10 min) or filter samples through a 0.22 µm filter. Be sure to select a s*olvent 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 HiTrap™ Desalting on Sephadex™ G–25.

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

Table 1. Recommended buffers for various pH intervals.

pH intervals	Buffer	Concentration[1]	Anion[2]	^{pKa} (25 °C)	dpKa[3] / dT(°C)
4.5–5.0	N-methylpiperazine	20 mM	Cl^-	4.75	-0.015
5.0–6.0	piperazine	20 mM	Cl^-	5.68	-0.015
			$HCOO^-$		
5.5–6.0	L-histidine	20 mM	Cl^-	6.15	
5.8–6.4	bis-Tris	20 mM	Cl^-	6.46	-0.017
6.4–7.3	bis-Tris propane	20 mM	Cl^-	6.80	
7.3–7.7	triethanolamine	20 mM	Cl^-	7.76	-0.020
7.5–8.0	Tris	20 mM	Cl^-	8.06	-0.028
8.0–8.5	N-methyldiethanolamine	20 mM	SO_4^{2-}	8.54	-0.028
		50 mM	Cl^-		
		50 mM	OAc^-		
8.4–8.8	diethanolamine	20 mM at 8.4 50 mM at 8.8	Cl^-	8.88	-0.025
8.5–9.0	1.3-diaminopropane	20 mM	Cl^-	8.64	-0.031
9.0–9.5	ethanolamine	20 mM	Cl^-	9.50	-0.030
9.5–9.8	piperazine	20 mM	Cl^-	9.73	-0.026
9.8–10.3	1.3-diaminopropane	20 mM	Cl^-	10.62	-0.026

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. Wash with 10 ml of eluent B (high ionic strength).
3. Equilibrate with 5 ml of 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. The most convenient and reproducible sample injection is via the Amersham Biosciences 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 , 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 1–5 below. More frequent washing may be necessary if complex samples are applied.

Steps 1–7 are guidelines. Use knowledge about possible contamination to evaluate which cleaning method is suitable.

1. Connect the column **inlet** to the detector. Set the sensitivity to 2.0 AUFS.
2. Make sure there is no space between the gel and adaptor. Start a reversed flow at a rate of 0.25–0.50 ml/min. Carry out steps 3–5 in sequence, ensuring each time that the monitored peaks are identical in size before proceeding to the next step.
3. Inject 500 µl 2 M NaCl solution. Rinse with water or buffer.
4. Inject 500 µl 2 M NaOH solution. Rinse with water or buffer.
5. Inject 500 µl 75% acetic acid or 1% TFA. Rinse with water or buffer and equilibrate.
6. 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 must be at a temperature of 37 °C (98.6 °F). After the enzymatic cleaning, perform the chemical scrubbing (steps 1–5) again.
7. 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. 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 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. Haemoglobin (Sigma type 4, human) is a good test protein, since it is coloured and readily available.

1. Dissolve the haemoglobin, 10 mg/ml, in 20 mM diethanolamine, pH 8.5.
2. Apply approximately 100 µl of the haemoglobin solution to the column.
3. Elute with a 20 mM diethanolamine solution 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.

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 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 height (ml)

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

Sample:	20 µl of triglycine, 0.05 mg/ml
Eluent:	0.01 M Tris, pH 8.9
Flow rate:	0.5 ml/min
Detector:	UV-M, 214 nm, 0.05 AUFS
Chart speed:	3 cm/min

The number of theoretical plates per metre (H^{-1}) should be more than 25 000.

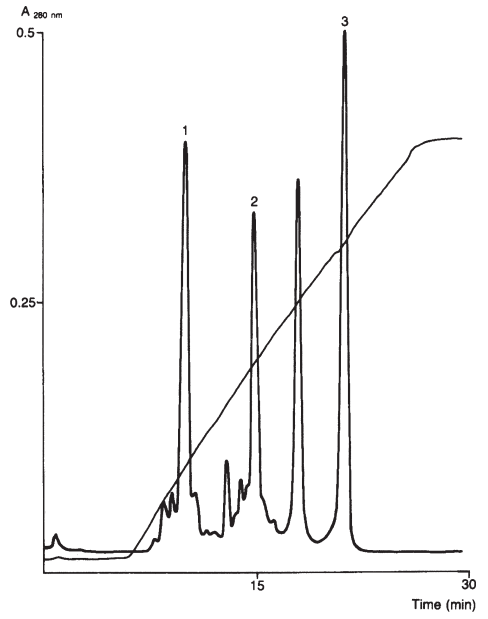

Fig 3. Typical chromatogram from a function test of Mono Q HR 5/5.

Function test

An alternative to the efficiency test to check column performance is the function test described here.
A typical chromatogram from a function test is described in figure 3.

Sample:	250 µl solution containing 1. Transferrin, 1 mg/ml 2. Ovalbumin, 2 mg/ml 3. ß-lactoglobulin, 2 mg/ml
Buffer A:	0.02 M Piperazine, pH 6.0
Buffer B:	0.02 M Piperazine, 0.3 M NaCl, pH 6.0
Flow rate:	1 ml/min
Gradient:	0.5–100% B in 20 min
Detector:	UV-M, 280 nm, 0.5 AUFS
Chart speed:	0.5 cm/min

Storage and prevention of microbial growth

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

Scaling up

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

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

Further information

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

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

For scaling up, there are two column sizes available:

Column	Bed volume	Code No.
Mono Q HR 10/10	8 ml	17-0556-01
Mono Q HR 16/10	20 ml	17-0506-01

Spare parts and accessories

Designation	Code No.	No. per pack
Top assembly HR 5	18-6352-01	1
Filter HR 5	18-6353-01	10
Bottom assembly HR 5	18-6351-01	1
Tubing connectors*	19-7476-01	5
Protective jacket HR 5/5	19-7447-01	1
Capillary tubing (o.d. 1.8 mm, i.d. 0.5 mm)	19-7477-01	2 m
Prefilter	19-5084-01	1
Filters + O-rings (prefilter)	19-5082-01	5+2
Flanging/Start-Up kit		
120 V	19-5079-01	1
220 V	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	18-1023-85	
Solvent resistant O-ring (for the Superloop)	18-6300-01	3
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
PD-10	17-0851-01	30
Fast Desalting Column HR 10/10	17-0591-01	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 Crawford Fitting
Valco is a trademark of Valco Instrument Co. Inc.

to order:

Asia Pacific Tel: +852 2811 8693 Fax: +852 2811 5251 **Australasia** Tel: +61 2 9899 0999 Fax: +61 2 9899 7511 **Austria** Tel: 01 576 0616 20 Fax: 01 576 0616 27 **Belgium** Tel: 0800 73 888 Fax: 03 272 1637 **Canada** Tel: 1 800 463 5800 Fax: 1 800 567 1008 **Central, East, South East Europe** Tel: +43 1 982 3826 Fax: +43 1 985 8327 **Denmark** Tel: 45 16 2400 Fax: 45 16 2424 **Finland & Baltics** Tel: +358 (0)9 512 3940 Fax: +358 (0)9 512 1710 **France** Tel: 0169 35 67 00 Fax: 0169 41 9677 **Germany** Tel: 0761 4903 401 Fax: 0761 4903 405 **Italy** Tel: 02 27322 1 Fax: 02 27302 212 **Japan** Tel: 81 3 5331 9336 Fax: 81 3 5331 9370 **Latin America** Tel: +55 11 3933 7300 Fax: +55 11 3933 7315 **Middle East and Africa** Tel: +30 (1) 96 00 687 Fax: +30 (1) 96 00 693 **Netherlands** Tel: 0165 580 410 Fax: 0165 580 401 **Norway** Tel: 2318 5800 Fax: 2318 6800 **Portugal** Tel: 21 417 7035 Fax: 21 417 3184 **Russia & other C.I.S. & N.I.S.** Tel: +7 (095) 232 0250,956 1137 Fax: +7 (095) 230 6377 **South East Asia** Tel: 60 3 8024 2080 Fax: 60 3 8024 2090 **Spain** Tel: 93 594 49 50 Fax: 93 594 49 55 **Sweden** Tel: +46 18 612 19 00 Fax: +46 18 612 19 10 **Switzerland** Tel: 01 802 81 50 Fax: 01 802 81 51 **UK** Tel: 0800 616 928 Fax: 0800 616 927 **USA** Tel: +1 800 526 3593 Fax: +1 877 295 8102

Produced by Wikströms, Sweden 1020972. 08.2002
Printed matter. Licence 341 051

GE imagination at work