GE Healthcare

Instructions 71-5017-88 AC **Ion Exchange Columns**

Mono Q 5/50 GL and Mono S 5/50 GL

Quick information

Mono Q™ 5/50 GL and Mono S™ 5/50 GL are Tricorn™ high performance columns. The columns are pre-packed glass columns for high performance ion exchange chromatography of proteins, peptides, polynucleotides and other biomolecules.

The columns are supplied with two union M6 female/1/16" male for connection to FPLC™ System, two fingertight connector 1/16" for connecting 1/16" tubing to column and ÄKTA, two stop plugs 1/16" male to seal the column (attached to column when delivered) and instruction.

Column data

Matrix	Polystyrene/divinyl benzene	
Bead form	Rigid, spherical, porous monodisperse	
Particle size	10 µm	
Column dimensions	5 × 50 mm	
Bed volume	1 ml	
Average loading capacity	50 mg	
(will vary depending on sample and loading conditions)		
pH stability		
regular use	2–12	
cleaning	1–14	
Temperature		
operating	4 to 40 °C	
Flow rate (water at room temperature)		
recommended	0.5–3.0 ml/min	
maximum	3 ml/min	
Pressure over column		
maximum	4 MPa, 40 bar, 580 psi	
	Mono Q	**Mono S**
Type of exchanger	Strong anion	Strong cation
Charged group	$-CH_2-N^+(CH_3)_3$	$-CH_2-SO_3^-$
Ionic capacity	0.27–0.37 mmol	0.12–0.15 mmol
	Cl^-/ml medium	H^+/ml medium

Note: Before connecting the column to a chromatography system, start the pump and remove all air and debris in the system, particularly in the tubing and valves.

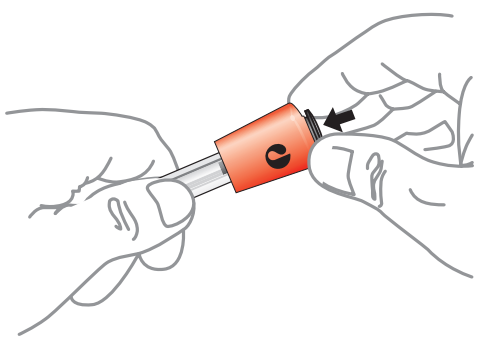

Fig 1. Illustration of how to lock the upper adapter. The locking ring (black) must be in down position to prevent uncontrolled adjustment of the column's bed height.

First-time use

Equilibrate the column for first-time use or after long-term storage as follows:

a) 5 column volumes (CV) distilled water at 1 ml/min at room temperature.
b) 5 CV start buffer at 2 ml/min at room temperature.
c) 5 CV elution buffer at 2 ml/min at room temperature.
d) 5 CV start buffer at 2 ml/min at room temperature.

Try these conditions first

Start buffer (Mono Q)*: 20 mM Tris-HCl, pH 8.0
Elution buffer (Mono Q)*: 20 mM Tris-HCl + 1.0 M NaCl, pH 8.0
Start buffer (Mono S)*: 20 mM 2-[N-morpholino] ethanesulphonic acid (MES), pH 6.0
Elution buffer (Mono S)*: 20 mM MES + 1.0 M NaCl, pH 6.0

* Users of ÄKTA™ design system may select one of the buffer recipes recommended for anion exchange chromatography at pH 8 or cation exchange chromatography at pH 6.

Separation by gradient elution

Flow: 2 ml/min at room temperature

1. Equilibrate column with 5–10 column volumes (CV) of start buffer or until baseline, eluent pH and conductivity are stable.
2. Adjust the sample to the chosen starting pH and ionic strength and apply to the column (see sample recommendations).
3. Wash with 5–10 CV of start buffer or until the baseline, the eluent pH and the conductivity are stable i.e. when all unbound material has washed through the column.
4. Begin elution using a gradient volume of 10–20 CV and an increasing ionic strengt up to 0.5 M NaCl (50% elution buffer).
5. Wash with 2–5 CV of 1 M NaCl (100% elution buffer) to elute any remaining ionically-bound material.
6. Requilibrate with at least 5–10 CV of start buff er or until eluent pH and conductivity reach the required values.

   Read the section "Optimization" for information about how to optimize a separation.

Buffers and solvent resistance

Recommended to have an on-line filter upstream of the injection valve. Buffers and solvents with increased viscosity will affect the back-pressure and flow rate. De-gas and filter all solutions through a 0.22 µm filter.

Daily use

All commonly used aqueous buffers, pH 2–12
Urea, up to 8 M
Guanidine hydrochloride, up to 6 M
Acetonitrile, up to 30% in aqueous buffers
Non-ionic detergents
Cationic detergents (Mono Q)
Anionic detergents (Mono S)

Cleaning

Acetonitrile, up to 100%
Sodium hydroxide, up to 2 M
Ethanol, up to 100%
Methanol, up to 100%
Acetic acid, up to 75%
Isopropanol, up to 100%
Hydrochloric acid, up to 1 M
1% Trifluoroacetic acid

Avoid:

Oxidizing agents
Anionic detergents (Mono Q)
Cationic detergents (Mono S)

Sample recommendations

Net charge of target molecule	negative (Mono Q), positive (Mono S)
Recommended initial sample load	≤ 45 mg
Preparation	Dissolve the sample in start buffer, filter through a 0.22 µm filter or centrifuge at 10 000 × g for 10 min

In-depth information

Delivery/storage

The column is delivered in degassed 20% ethanol sealed with two stop plugs to prevent the column from drying out. For column storage, wash with 5 column volumes of distilled water followed by 5 column volumes of 20% ethanol. Degas the ethanol/water mixture thoroughly and apply at a low flow rate to avoid over-pressuring the column. Store at room temperature or, for long periods, store at +4° C to +8 °C. Ensure that the column is sealed well to avoid drying out. Do not freeze.

Choice of eluent

To avoid local disturbances in pH caused by buffering ions participating in the ion exchange process, select an eluent with buffering ions of the same charge as the substituent groups on the ion exchanger.

Choose the start buffer pH so that substances to be bound to the ion exchanger are charged, e.g. at least 1 pH unit above the isoelectric point for anion exchangers and at least 1 pH unit below the isoelectric point for cation exchangers. Figure 2 and Figure 3 list a selection of standard aqueous buffers.

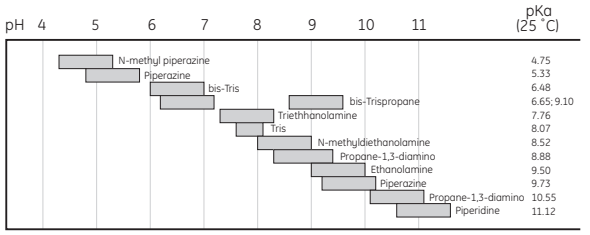

Fig 2. Recommended buffers for anion exchange chromatography.

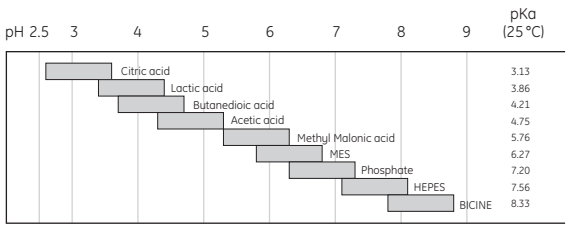

Fig 3. Recommended buffers for cation exchange chromatography.

Table 1 lists suggested volatile buffers that can be used in cases where the purified substance has to be freeze-dried.

Table 1. Volatile buffer systems.

pH	Substance
3.3–4.3; 4.8–5.8	Pyridine/formic acid
3.3–4.3; 9.3–10.3	Trimethylamine/formic acid
4.3–5.8	Pyridine/acetic acid
3.3–4.3; 8.8–9.8	Ammonia/formic acid
4.3–5.3; 8.8–9.8	Ammonia/acetic acid
5.9–6.9; 9.3–10.3	Trimethylamine/carbonate
5.9–6.9; 8.8–9.8	Ammonium carbonate/ammonia
4.3–5.3; 7.2–8.2	N-ethylmorpholine/acetate

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Optimization

Perform a first run as described in the section "Try these conditions first". If the results obtained are unsatisfactory, consider the following:

Action	Effect
Change pH/buffer salt (see Figure 1 and Figure 2 for buffers)	Changes selectivity, gives weaker/stronger binding.
Change salt, counter ions and/or co-ions	Changes selectivity.
Decrease the sample load	Improves resolution.
Decrease the flow rate	Improves resolution.
Change gradient slope	Shallower gradients improve selectivity but broaden peaks (decrease efficiency). A steeper gradient will sharpen peaks, but move them closer together.

For more information, please refer to the handbook "Ion exchange chromatography, Principles & Methods", which can be ordered from GE Healthcare or downloaded from our web site.

Cleaning

It is recommended to reverse the direction of flow during column cleaning so that contaminants do not need to pass through the entire length of the column.

Regular cleaning

Flow: 0.5 ml/min at room temperature

1. Wash with 2 column volumes (CV) of 2 M NaCl.
2. Wash with 4 CV of 1 M NaOH
3. Wash with at least 2 CV of 2 M NaCl
4. Rinse with at least 2CV of distilled water until the UV-baseline and the eluent pH are stable.
4. Wash with at least 4 CV of start buffer or storage buffer until pH and conductivity values have reached the required values.

More rigorous cleaning

Remove strongly hydrophobically bound proteins, lipoproteins and lipids by washing with 4 column volumes (CV) of 30% isopropanol or 70% ethanol at 0.25 ml/min. Remove precipitated proteins with 1 CV of 1 mg/ml pepsin in 0.5 M NaCl, 0.1 M acetic acid (leave overnight) or wash with 2 CV of 6 M Guanidine hydrochloride at 0.25 ml/min.

Depending on the nature of contaminant cleaning solution in the section "Buffers and solvent resistance" may be appropriate. After cleaning the column wash with at least 2 CV of distilled water and 4 CV of start buffer or storage buffer. For more information on how to clean your column, please refer to the handbook "Ion exchange chromatography & Chromatofocusing, Principles & Methods".

As an alternative to more rigorous cleaning or if column performance still not restored change the filter at the top of the column. (Since contaminants are introduced with the liquid flow, many of them are caught by the filter.) Instructions for changing the filter are supplied with the Filter Kit. Clean the column after filter change according to regular cleaning.

Troubleshooting

Symptom	Remedy
Increased back-pressure over the column	Reverse the flow direction and pump 5 ml elution buffer at a flow rate of 0.5 ml/min through the column. Return to normal flow direction and run for 5 minutes at 1 ml/min through the column. If high backpressure persists, clean the column.
Loss of resolution and/or decreased sample recovery	Clean the column according to the procedure described in the section "More rigorous cleaning".
Air in the column	Reverse the flow direction and pump 10 ml well de-gassed start buffer through the column at a flow rate of 0.5 ml/min.

Column performance control

Check the performance of the column when new by running the separation described in Figures 4 and 5. Figures 4 and 5 shows a typical chromatogram run on an optimized system. Since the system can profoundly affect the resolution, it is meaningful to compare runs done on the same system. Check the column at regular intervals and whenever you suspect a problem.

Function test of Mono Q 5/50 GL

Sample: 1. Conalbumin (3 mg/ml)
2. α-lactalbumin, bovine milk (4 mg/ml)
3. Soybean trypsin inhibitor (6 mg/ml)

Sample volume: 200 µl
Gradient: 0–100% elution buffer in 20 CV
Start buffer: 20 mM Tris-HCl, pH 7.0
Elution buffer: 20 mM Tris-HCl + 0.25 M NaCl, pH 7.0
Flow rate: 1.0 ml/min (room temperature)

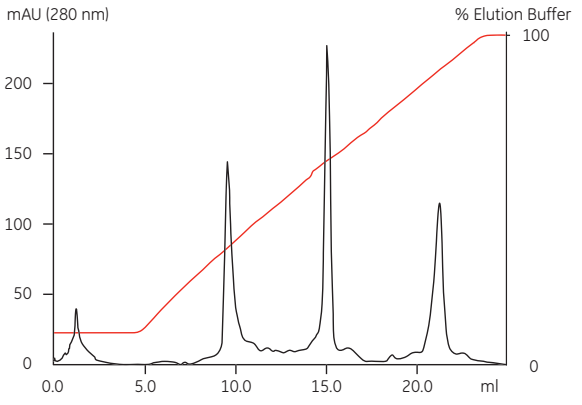

Fig 4. Typical chromatograms from a function test of Mono Q 5/50 GL.

Function test of Mono S 5/50 GL

Sample: 1. Ribonuclease A (1.5 mg/ml)
2. Cytochrome C (0.4 mg/ml)
3. Lysozyme (0.4 mg/ml)

Sample volume: 100 µl
Gradient: 0–100% elution buffer in 20 CV
Start buffer: 20 mM sodium phosphate, pH 6.8
Elution buffer: 20 mM sodium phosphate + 0.4 M NaCl, pH 6.8
Flow rate: 1.0 ml/min (room temperature)

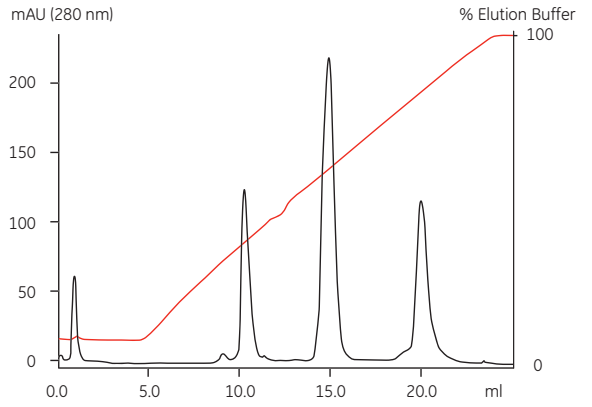

Fig 5. Typical chromatograms from a function test of Mono S 5/50 GL.

Ordering information

Product	No. per pack	Code No.
Mono Q 5/50 GL	1	17-5166-01
Mono S 5/50 GL	1	17-5168-01

Related products

Product	No. per pack	Code No.
Mono Q 10/100 GL	1	17-5167-01
Mono Q 4.6/100 PE	1	17-5179-01
Mono S 10/100 GL	1	17-5169-01
Mono S 4.6/100 PE	1	17-5180-01
HiTrap™ Desalting	5 × 5 ml	17-1408-01

Accessories

Product	No. per pack	Code No.
Tubing connectors:		
Fingertight connector 1/16" male	10	18-1112-55
Tricorn 5 filter kit*	1	18-1153-02
Filter Tool	1	18-1153-20
Union M6 female/1/16" male	8	18-1112-58
On-line filter (1/16")	1	18-1118-01
Handbook:		
Ion Exchange Chromatography & Chromatofocusing, Principles and Methods	1	11-0004-21

* includes top and bottom filters and O-rings, 5 of each.

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www.gehealthcare.com

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71-5017-88 AC 03/2006

Elanders Österåla 2006