Mono Q™ 10/100 GL and Mono S™ 10/100 GL

Quick information

Mono Q 10/100 GL and Mono S 10/100 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™, storage/shipping device attached to column for storage and shipping, two stop plugs 1/16" male to seal the column (one attached to column when delivered) and instruction.

Column data

Matrix	Polystyrene/divinyl benzene
Bead form	Spherical, porous monodisperse
Particle size	10 µm
Column dimensions	10 × 100 mm
Bed volume	8 ml
Average loading capacity	400 mg
(will vary depending on sample and loading conditions)	
pH stability	
regular use	2 to 12
cleaning	1 to 14
Temperature	
operating	4 °C to 40 °C
storage	4 °C to 30 °C
Flow rate (water at room temperature)	
recommended	2–6 ml/min
maximum	10 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	O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2-$N^+(CH_3)_3$	-O-CH_2-CHOH-CH2-O-CH_2-CHOH-CH_2-SO_3^-
Ionic capacity	0.27–0.37 mmol Cl^-/ml medium	0.12–0.15 mmol H^+/ml medium

First-time use

Before connecting the column to a chromatography system, ensure there is no air in the tubing and valves. Remove the storage/shipping device and the stop plug from the column. Check that the adapter is locked, see figure 1. Make sure that the column inlet is filled with liquid and connect it drop-to-drop to the system.

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

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

1. 5 column volumes (CV) distilled water at 2 ml/min at room temperature.
2. 5 CV starting buffer at 2 ml/min at room temperature.
3. 5 CV elution buffer at 4 ml/min at room temperature.
4. 5 CV starting buffer at 4 ml/min at room temperature.

Try these conditions first

Flow rate:	4 ml/min at room temperature
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

Separation by gradient elution

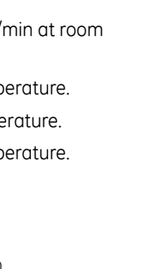
Flow: 4 ml/min

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 buffer or until eluent pH and conductivity reach the required values.

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

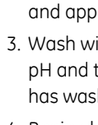
Sample recommendations

Net charge of target molecule	Negative (Mono Q), Positive (Mono S)
Recommended initial sample load	≤ 360 mg
Preparation	Dissolve the sample in start buffer, filter through a 0.22 µm filter or centrifuge at 10 000 × g for 10 min



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
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
Guanidine hydrochloride, up to 6 M
Trifluoroacetic acid, up to 1%

Avoid:

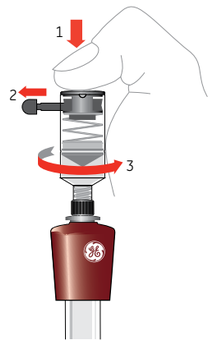
Oxidising agents
Anionic detergents (Mono Q)
Cationic detergents (Mono S)

In-depth information

Delivery/storage

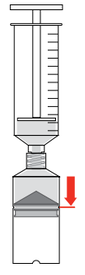
The column is delivered in degassed 20% ethanol with a storage/shipping device that prevents the column from drying out. If the column is to be stored for more than 2 days after use, wash the column with 4 column volumes (CV) of distilled water and then equilibrate with at least 4 CV of degassed 20% ethanol. Use a low flow rate, checking backpressure as the column equilibrates. Connect the storage/shipping device according to instructions "How to connect the storage/shipping device". Store at room temperature or, for long periods, store at 4 °C to 8 °C. Do not freeze.

The glass tube is coated with a protecting plastic film. Small quantities of air may occasionally be trapped between the glass and the film during manufacture. The resulting uneven surface does not affect column performance or durability.



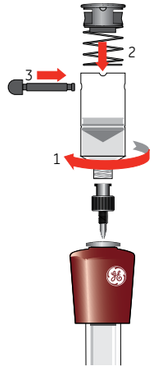
How to remove the storage/shipping device.

(1) Push down the spring-loaded cap
(2) Remove the locking pin
(3) Release the cap and unscrew the device



How to refill the storage/shipping device.

(1) Connect a syringe or pump to the storage/shipping device and fill with 20% ethanol over the mark on the device. Remove the syringe or pump connection.
(2) Tap out air bubbles and push the plunger to the mark on the device.



How to connect the storage/shipping device.

(1) Seal the bottom unit with a stop plug. Fill the column inlet and luer connctor with 20% ethanol and connect the filled storage/shipping device drop-to-drop to the top of the column.
(2,3) Mount the spring-loaded cap and secure it with the locking pin.

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.

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

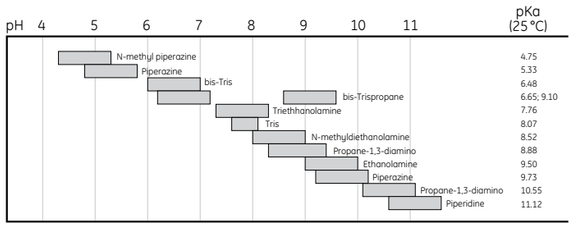


Fig. 2. Recommended buffers for anion exchange chromatography

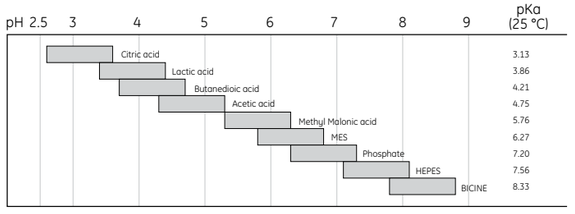


Fig. 3. Recommended buffers for cation exchange chromatography

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

Optimization

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

Action	Effect
Change pH/buffer salt (see Figure 2 and Figure 3 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 & Chromatofocusing, 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: 2 ml/min

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 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 2 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 2 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 40 ml elution buffer at a flow rate of 2 ml/min through the column. Return to normal flow direction and run for 10 minutes at 4 ml/min. If high back-pressure persists, clean the column.
Loss of resolution and/or decreased sample recovery	Clean the column.
Air in the column	Reverse the flow direction and pump 80 ml of well de-gassed starting buffer through the column at a flow rate of 2 ml/min.

Column performance control

Check column performance at regular intervals and whenever you suspect a problem. Check the function of the column when new by running the separation described in Figure 4 and 5. Compare the resulting chromatogram with later runs under the same conditions.

Function test of Mono Q 10/100 GL

Sample:	1. Conalbumin (3 mg/ml) 2. α-lactalbumin, bovine milk (4 mg/ml) 3. Soybean trypsin inhibitor (6 mg/ml)
Sample volume:	500 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:	4 ml/min (room temperature)

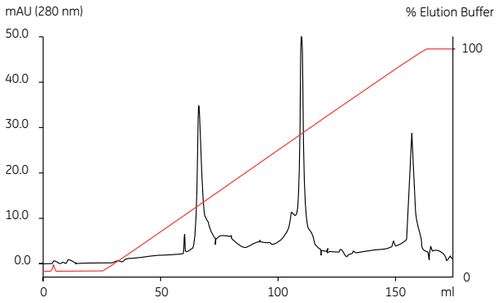


Fig. 4. Typical chromatograms from a function test of Mono Q 10/100 GL

Function test of Mono S 10/100 GL

Sample:	1. Ribonuclease A (6 mg/ml) 2. Cytochrome C (1.5 mg/ml) 3. Lysozyme (1.5 mg/ml)
Sample volume:	500 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:	4 ml/min (room temperature)

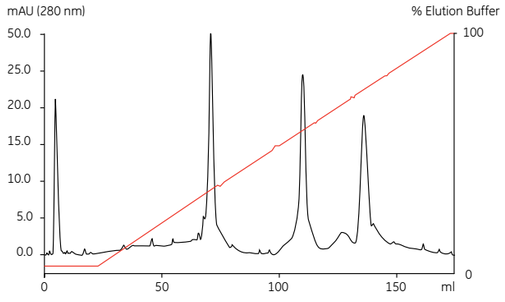


Fig. 5. Typical chromatograms from a function test of Mono S 10/100 GL

Ordering information

Designation	No. per pack	Code No.
Mono Q 10/100 GL	1	17-5167-01
Mono S 10/100 GL	1	17-5169-01

Related products

Designation	No. per pack	Code No.
Mono Q 5/50 GL	1	17-5166-01
Mono Q 4.6/100 PE	1	17-5179-01
Mono S 5/50 GL	1	17-5168-01
Mono S 4.6/100 PE	1	17-5180-01
HiTrap™ Desalting	5 × 5 ml	17-1408-01
HiPrep™ 26/10 Desalting	1	17-5087-01

Accessories

Designation	No. per pack	Code No.
Fingertight connector 1/16" male	10	18-1112-55
Tricorn 10 filter kit*/**	1	29-0536-12
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
1/16" male to Luer female	2	18-1112-51
Storage/shipping device	1	18-1176-43
Handbook: Ion Exchange chromatography & Chromatofocusing, Principles & Methods	1	11-0004-21

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

** Do not store exposed to daylight.

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