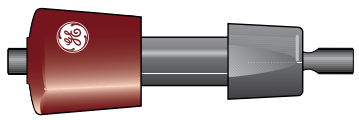
Mini Q™ 4.6/50 PE and Mini S™ 4.6/50 PE



Quick information

Mini Q 4.6/50 PE and Mini S 4.6/50 PE are Tricorn™ high performance columns. The columns are pre-packed PEEK columns for high performance ion exchange chromatography of proteins, peptides, polynucleotides and other biomolecules.

Column data

Matrix	Polystyrene/divinyl benzene
Bead form	Spherical, non-porous monodisperse
Particle size	3 µm
Column dimensions	4.6 × 50 mm
Bed volume	0.8 ml
Maximum loading capacity (will vary depending on sample and loading conditions)	3–5 mg
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	0.5–2.0 ml/min
maximum	2 ml/min
Pressure over column	
maximum	18 MPa, 180 bar, 2610 psi

	Mini Q	Mini S
Type of exchanger	Strong anion	Strong cation
Charged group	$O\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}N^+(CH_3)_3$	$-O\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}SO_3^-$
Ionic capacity	0.085–0.115 mmol Cl^-/ml medium	0.018–0.034 mmo H^+/ml medium

First-time use

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

a) 4 ml distilled water at 0.5 ml/min at room temperature.

b) 4 ml start buffer at 0.5 ml/min at room temperature.

c) 4 ml elution buffer (including at least 1 M NaCl) at 0.8 ml/min at room temperature.

d) 4 ml start buffer at 0.8 ml/min at room temperature.

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

Try these conditions first

Flow rate:	0.8 ml/min at room temperature
Gradient:	0–100% elution buffer in 20 column volumes (CV)
Start buffer (Mini Q)*:	20 mM Tris-HCl, pH 8.0
Elution buffer (Mini Q)*:	20 mM Tris-HCl + 1.0 M NaCl, pH 8.0
Start buffer (Mini S)*:	20 mM 2-[N-morpholino] ethanesulphonic acid (MES), pH 6.0
Elution buffer (Mini 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.

Equilibration between runs:

Proceed according to steps c) and d) in the section "First-time use". Extended equilibration may be needed if detergents are included in the eluent.

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

Buffers and solvent resistance

Install an on-line filter upstream of the injection valve. Buffers and solvents with increased viscosity will affect the backpressure 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 (Mini Q)
Anionic detergents (Mini S)

Cleaning

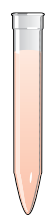
Acetonitrile, up to 100%
Sodium hydroxide, up to 2 M
Ethanol, up to 100%
Methanol, up to 100%
Acetic acid, up to 75%
Isopropanol, up to 100%
Hydrochloric acid, up to 1 M
Guanidine hydrochloride, up to 6 M

Avoid

Unfiltered solutions
Oxidizing agents
Anionic detergents (Mini Q)
Cationic detergents (Mini S)

Sample recommendations

Net charge of target molecule	negative (Mini Q), positive (Mini S)
Recommended initial sample load	≤ 0.7 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

Mini Q 4.6/50 PE is delivered in 20% ethanol. Mini S 4.6/50 PE is delivered in 20% ethanol containing 0.2 M sodium acetate. If the column is to be stored for more than two days after use, wash the column with 4 ml distilled water and then equilibrate Mini Q 4.6/50 PE with at least 4 ml 20% ethanol or Mini S 4.6/50 PE with at least 4 ml 20% ethanol containing 0.2 M sodium acetate.

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 1 and Figure 2 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.

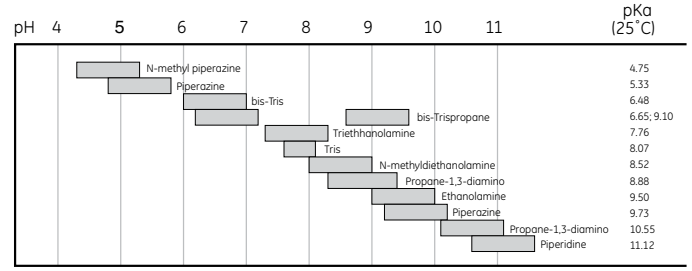


Fig 1. Recommended buffers for anion exchange chromatography.

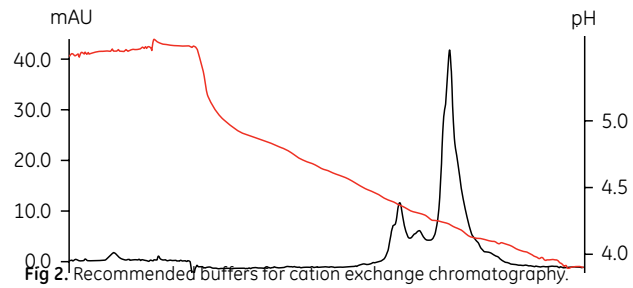


Fig 2. Recommended buffers for cation exchange chromatography.

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

Optimisation

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 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 and Chromatofocusing, which can be ordered from GE Healthcare, or to the "Method Handbook" supplied with each ÄKTA design system.

Cleaning-in-place (CIP)

Regular cleaning:

Wash the column with 1.5 ml 2 M NaCl after each run to elute material still bound to the column. If detergents have been used, rinse the column with 4 ml distilled water followed by 1.5 ml 2 M NaCl. Re-equilibrate the column until the UV baseline and conductivity are stable (usually at least 10 CV).

More rigorous cleaning:

Reverse the flow direction and run the following sequence of solutions at a flow rate of 0.2 ml/min:

1. 3 ml 1 M NaCl
2. 3 ml 1 M NaOH
3. 3 ml 1 M HCl
4. 3 ml 1 M NaCl

Note: Always rinse with at least 1.5 ml distilled water between each step.

Do not store the column in 1 M HCl or 1 M NaOH.

Depending on the nature of the contaminants, the following cleaning solutions may also be appropriate:

30% Isopropanol

30% Acetonitrile

2 M NaOH containing 1 M NaCl

75% Acetic acid containing 1 M NaCl

1% Trifluoroacetic acid

Note: Always rinse with at least 1.5 ml distilled water after any of the above cleaning solutions has been used.

If column performance is still not restored, inject a solution of 1 mg/ml pepsin in 0.1 M acetic acid including 0.5 M NaCl and leave overnight at room temperature or one hour at 37°C. Depending on the contaminant, other enzymes may also be used, e.g. DNase. After enzymatic treatment, repeat steps 1–4 in the "More rigorous cleaning" described above. After cleaning, equilibrate the column in the normal flow direction before use.

DO NOT OPEN THE COLUMN!

Troubleshooting

Symptom	Remedy
Increased back-pressure over the column	Reverse the flow direction and pump 4 ml elution buffer at a flow rate of 0.1 ml/min through the column. Return to normal flow direction and run for 10 minutes at a flow rate of 0.5 ml/min. If high back-pressure 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 of well de-gassed start buffer through the column at a flow rate of 0.2 ml/min.

Column performance control

Check the performance of the column when new by running the separation described in fig. 3 and 4. Figure 3 and 4 shows a typical chromatogram on an optimised system. Since the system can profoundly affect the resolution it is most meaningful to compare runs done on the same system. Check the column at regular intervals and whenever you suspect a problem.

Column Mini Q 4.6/50 PE

Sample:
1. a-Amylase (48 µg/ml)
2. a-Lactalbumin (72 µg/ml)
3. Trypsin inhibitor (0.43 mg/ml)

Sample volume: 200 µl
Gradient: 0–100% elution buffer in 12 CV
Start buffer: 20 mM Tris-HCl, pH 7.5
Elution buffer: 20 mM Tris-HCl+ 0.25 M NaCl, pH 7.5
Flow rate: 0.83 ml/min (room temperature)

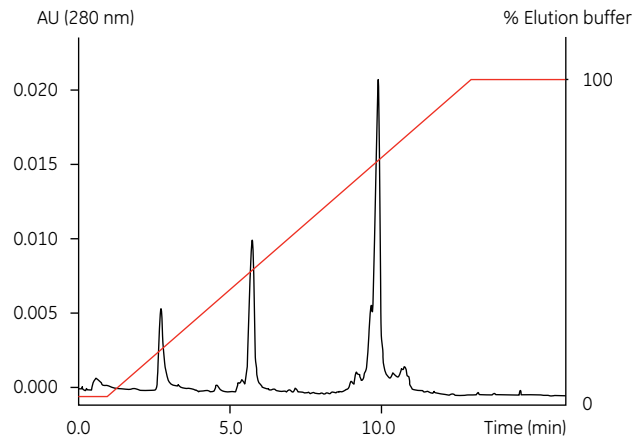


Fig 3. Typical chromatograms from a function test of Mini Q 4.6/50 PE.

Column Mini S 4.6/50 PE

Sample:
1. a-Chymotrypsinogen (25 µg/ml)
2. Ribonuclease A (75 µg/ml)
3. Lysozyme (25 µg/ml)

Sample volume: 200 µl
Gradient: 0–100% elution buffer in 12 CV
Start buffer: 20 mM Sodium acetate, pH 5.0
Elution buffer: 20 mM Sodium acetate + 0.4 M NaCl, pH 5.0
Flow rate: 0.83 ml/min (room temperature)

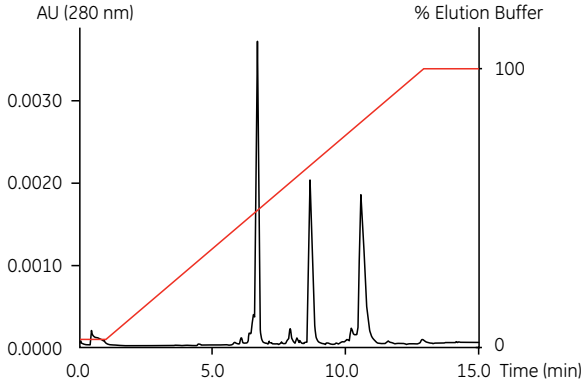


Fig 4. Typical chromatograms from a function test of Mini S 4.6/50 PE.

Ordering information

Products	No. per pack	Code No.
Mini Q 4.6/50 PE	1	17-5177-01
Mini S 4.6/50 PE	1	17-5178-01

Related products

HiTrap™ Desalting	5 × 5 ml	17-1408-01

Accessories

Tubing connectors:		
Fingertight connector 1/16" male	10	18-1112-55
Union M6 female/1/16" male	8	18-1112-58
On-line filter (1/16")	1	18-1118-01
Handbook: Ion Exchange chromatography and Chromatofocusing	1	11-0004-21

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