Mono Q 4.6/100 PE and **Mono S** 4.6/100 PE

Instructions for Use

Quick information

Mono Q™ 4.6/100 PE and Mono S™ 4.6/100 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.

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	4.6 x 100 mm
Bed volume	1.7 ml
Average loading capacity[1]	85 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	0.5–3.0 ml/min
maximum	3 ml/min
Max. pressure	4.0 MPa, 40 bar, 580 psi

	Mono Q	**Mono S**
Type of exchanger	Strong anion	Strong cation
Charged group	-CH2-N+(CH3)3	-CH2-SO3 -
Ionic capacity	0.27–0.37 mmol	0.27–0.37 mmol
	Cl-/mL medium	H+/ml medium

[1] Average loading capacity will vary

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.*

First-time use

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

Step	**Action**
1	5 column volumes (CV) distilled water at 1 mL/min at room temperature.
2	5 CV start buffer at 2 mL/min at room temperature.
3	5 CV elution buffer at 2 mL/min at room temperature.
4	5 CV start buffer at 2 mL/min at room temperature.

Try these conditions first

Start buffer (Mono Q) [1]:	20 mM Tris-HCl, pH 8.0
Elution buffer (Mono Q)[1]:	20 mM Tris-HCl + 1.0 M NaCl, pH 8.0
Start buffer (Mono S)[1]:	20 mM 2-[N-morpholino] ethanesulphonic acid (MES), pH 6.0
Elution buffer (Mono S)[1]:	20 mM MES + 1.0 M NaCl, pH 6.0

[1] Users of the ÄKTA design system may select one of the buffer recipes recommended for anion exchange chromatograph at pH 8 or cation exchange chromatography at pH 6.

Separation by gradient elution

Flow: 2 mL/min at room temperature

Step	**Action**
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.

Step	Action
4	Begin elution using a gradient volume of 10–20 CV and an increasing ionic strength 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 on page 3* 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. Degas 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	≤ 80 mg	
Preparation	Dissolve the sample in start buffer, filter through a 0.22 µm filter or centrifuge at 10 000 x 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 overpressuring the column. Store at 4 °C to 30 °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. *Fig.1* below and *Fig. 2, on page 2* list a selection of standard aqueous buffers. *Table 1, on page 2* lists suggested volatile buffers that can be used in cases where the purified substance has to be freeze-dried.

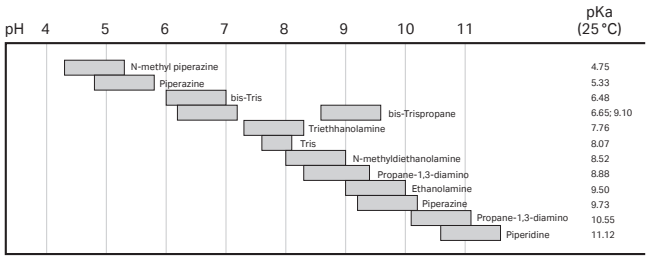

Fig 1. Recommended buffers for anion exchange chromatography

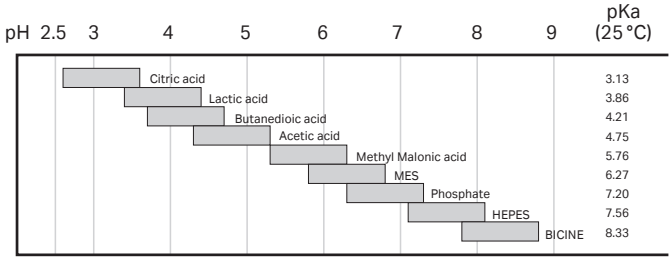

Fig 2. Recommended buffers for cation exchange chromatography

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

Optimization

Perform a first run as described in the section *Try these conditions first, on page 1* If the results obtained are unsatisfactory, consider the following:

Action	Effect
Change pH/buffer salt (see *Fig. 1, on page 2* and *Fig. 2, on page 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 & Chromatofocusing, Principles and Methods*, which can be ordered from Cytiva 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: 0.2 ml/min at room temperature

Step	Action
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.
5	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 0.1 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 0.1 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 and Methods".

IMPORTANT
DO NOT OPEN THE COLUMN!

Troubleshooting

Symptom	Remedy
Increased back-pressure over the column	Reverse the flow direction and pump 8 mL elution buffer at a flow rate of 0.5 mL/min through the column. Return to normal flow direction and run for 10 minutes at a flow rate of 1 mL/min. 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 20 ml well de-gassed start buffer through the column at a flow rate of 0.5 ml/min

Column performance control

Check the function of the column when new by running the separation described in *Fig. 3* and *Fig. 4*. Figures 3 and 4 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 Mono Q 4.6/100 PE

Sample:	1. Conalbumin (3 mg/mL) 2. α-lactalbumin (4 mg/mL) 3. Soybean trypsin inhibitor 1 (6 mg/mL)
Sample volume:	200 µL
Gradient:	0–100% elution buffer in 12 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 mL/min (room temperature)

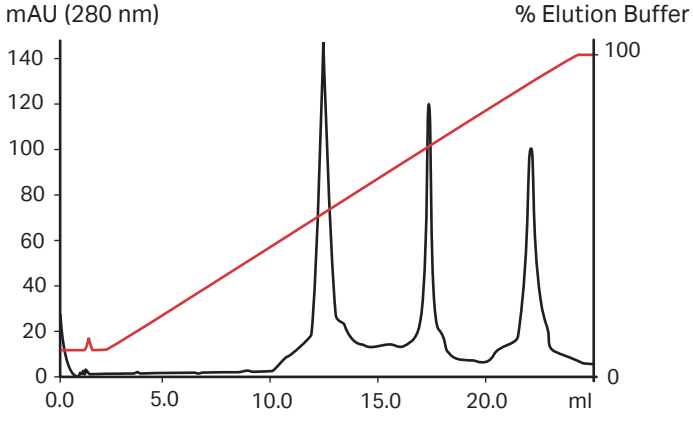

Fig 3. Typical chromatograms from a function test of Mono Q 4.6/100 PE

Function test Mono Q 4.6/100 PE

Sample:	1. Ribonuclease A (1.5 mg/mL) 2. Cytochrome C (0.4 mg/mL) 3. Lysozyme (0.4 mg/mL)
Sample volume:	200 µl
Gradient:	0–100% elution buffer in 12 CV
Start buffer:	20 mM Tris-HCl, pH 6.8
Elution buffer:	20 mM Tris-HCl + 0.4 M NaCl, pH 6.8

Flow rate: 1 mL/min (room temperature)

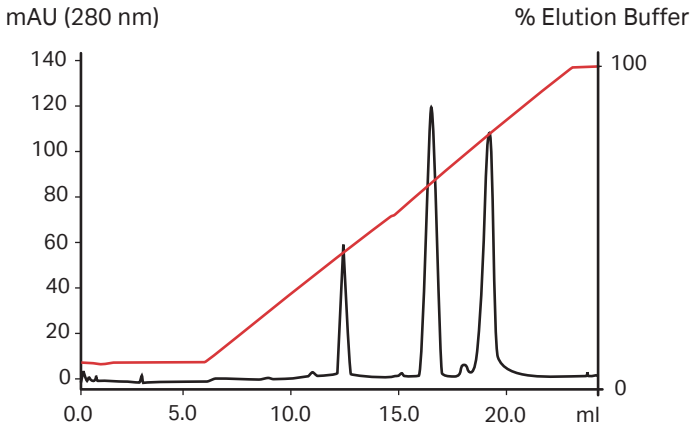

Fig 4. Typical chromatograms from a function test of Mono S 4.6/100 PE

Ordering information

Designation	No. per pack	Code No
Mono Q 4.6/100 PE	1	17-5179-01
Mono S 4.6/100 PE	1	17-5180-01

Related products

Designation	No. per pack	Code No
Mono Q 5/50 GL	1	17-5166-01
Mono Q 10/100 GL	1	17-5167-01
Mono S 5/50 GL	1	17-5168-01
Mono S 10/100 GL	1	17-5169-01
HiTrap™ Desalting	5 x 5mL	17-1408-01

Accessories

Designation	No. per pack	Code No.
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 & Chromatofocusing* Principles and Methods	1	11-0004-21

Visit us at:

www.cytivalifesciences.com

cytiva.com

71501790 AF V:6 11/2020