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 sightlight connector 1/16” for connecting 1/16” tubing to column and AKTA™ 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 size: Polyethylene beads
- Bed form: Spherical, porous monodisperse
- Porous size: 15 µm
- Column dimensions: 16 x 300 mm
- Bed volume: 10 ml
- Average loading capacity: 400 mg
- Non volatile depending on sample and loading conditions
- pH stability: 1) Regular use 2) Cleaning 3) Storage
- Temperature: 4) Operating 5) Storage
- Flow rate at room temperature recommended: 2-3 ml/min
- Pressure over column maximum: 6) 200 psi above 8 psi
- Type of exchanger: Mono Q Strong anion
- Charged group: C0-C4, CH3OH, CH3OH, CH3OH, CH3OH, CH3OH, CH3OH, CH3OH, CH3OH
- Ionic capacity: 1.0 mEq/ml medium

Mono Q

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.

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 NaCl, pH 8.0
Elution buffer (Mono Q): 20 mM Na+ - Cl- + 0.5 M NaCl, pH 8.0
Start buffer (Mono S): 20 mM NaCl, pH 6.0
Elution buffer (Mono S): 20 mM Na+ - Cl- + 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 slope to the chosen starting pH and intensity of the start buffer and until baseline, the eluent pH and conductivity are stable i.e. when all unbound material has washed through the column.
3. Begin elution using a gradient volume of 10–20 CV and an increasing ionic strength up to 0.5 M NaCl (50% elution buffer).
4. Wash with 2–5 CV of 1 M NaCl (100% elution buffer) to elute any remaining ionically-bound material.
5. Equilibrate 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.

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 a 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 ºC to 8 ºC. 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.

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% Tris

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

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.

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)

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

How to re-flip 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) Top up air bubbles and push the plunger to the mark on the device.

Table 1. Volatile buffer systems
<table>
<thead>
<tr>
<th>pH</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>Bis-Tris</td>
</tr>
<tr>
<td>5.0</td>
<td>Bis-Tris</td>
</tr>
<tr>
<td>6.0</td>
<td>Bis-Tris</td>
</tr>
<tr>
<td>7.0</td>
<td>Bis-Tris</td>
</tr>
<tr>
<td>8.0</td>
<td>Bis-Tris</td>
</tr>
<tr>
<td>9.0</td>
<td>Bis-Tris</td>
</tr>
<tr>
<td>10</td>
<td>Bis-Tris</td>
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<tr>
<td>11</td>
<td>Bis-Tris</td>
</tr>
<tr>
<td>12</td>
<td>Bis-Tris</td>
</tr>
</tbody>
</table>

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 height.

Fig. 2. Recommended buffers for anion exchange chromatography

Fig. 3. Recommended buffers for cation exchange chromatography
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 | Changes selectivity, gives weaker/stronger binding.
(see Figure 2 and Figure 3 for buffers)
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 NaOH 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.

Loss of resolution and/or decreased sample recovery

Air in the column

Reverse the flow direction and pump 80 ml de-gassed start buffer through the column at a flow rate of 2 ml/min.

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.
Clean the column.

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.

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.

Flow rate: 2 ml/min.

Reverse the flow direction and pump 80 ml de-gassed start buffer through the column at a flow rate of 2 ml/min.

Wash with at least 2 CV of 2 M NaCl until the UV-baseline and flow rate are stable.

Wash with 4 CV of 1 M NaOH.

Wash with 2 column volumes (CV) of 2 M NaCl.

Decreased sample recovery

Increased back-pressure over the column

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