Desalination and buffer exchange of biomolecules by gel filtration/size exclusion chromatography

ROTI®Dex columns for chromatography using syringe, pump or FPLC systems

ROTI®Dex provides a gel filtration matrix of epichlorohydrin-crosslinked dextran. This enables a form of group separation by size exclusion chromatography for the removal of salts and other low molecular weight factors from protein or nucleic acid solutions

The prepacked ROTI®Dex FPLC columns are used for purification and desalting of sample volumes between 0.05 and 1.5 ml. Biomolecules >5 kDa or >10 bp and particles >2 nm are purified. The fractionation range for proteins/peptides/biomolecules is between 1 and 5 kDa, with the highest resolution (most efficient separation) achieved between biomolecules >5 kDa and <1 kDa.

The column bed consists of ROTI®Dex-25 Superfine. It is a spherical, porous gel filtration medium consisting of dextran cross-linked with epichlorohydrin. The medium was pre-swollen in 20% ethanol.
For further information on ROTI®Dex, please refer to our Technical Information Brochure.

In the following you will find our recommended protocol and other important instructions for use of the prepacked ROTI®Dex FPLC columns.
This protocol is only a guide and should be adapted according to your specific needs.

Technical data of the ROTI®Dex FPLC columns:

Order No.	**21C6.1**	**21C6.2**	**21C7.1**	**21C7.2**	**21C7.3**
Package size	5 Pcs.	100 Pcs.	5 Pcs.	25 Pcs.	100 Pcs.
Matrix	ROTI®Dex 25 Superfine				
Particle size (wet)	40 - 110 µm				
Gel bed volume	1 ml		5 ml		
Sample volume	0.05 – 0.3 ml		0.1 – 1.5 ml		
Pressure max.	3 bar (0.3 MPa)				
Flow rate max.	3 ml/min		10 ml/min		
Recommended flow rate	0.5 - 2 ml/min		1 - 5 ml/min		
Outlet port	10–32 (1/16”) male				
Inlet port	10–32 (1/16”) female				
Column size	0.7 cm inner diameter x 2.5 cm height		1.6 cm inner diameter x 2.5 cm height		

1. Choose the right buffer

One of the advantages of size exclusion chromatography is the easy and fast exchange of the buffer solution in which your biomolecules are dissolved. So choose the buffer in which you need your sample for further downstream applications, or use the buffer in which your sample is already located, if you only want to purify it.

The chemically very stable column matrix, allows purification in the presence of essential ions, cofactors, detergents, urea, guanidine hydrochloride, etc.. Thus, all common aqueous buffers can be used for desalting/buffer exchange. Often a buffer with 25 to 50 mM concentration of the buffer substance (e.g. sodium phosphate, Tris-HCL, etc.) and a pH between 7 and 8 is sufficient. An additional salt concentration of at least 25 mM (usually NaCl) is recommended to avoid possible ionic interactions (seen as delays in peak elution or as broad peaks). Volatile buffers such as 100 mM ammonium acetate or 100 mM ammonium bicarbonate can be used if the presence of sodium chloride must be avoided.
Proteins require saline solutions, but not too much. Thus, avoid the use of pure water, as well as salt concentrations above 1 M.

Use high quality water and chemicals. Solutions should be filtered through 0.45 µm or 0.22 µm filters. Degas buffers before each gel filtration to avoid air bubbles. Buffers are automatically degassed when filtered under vacuum. Buffers and columns must be at the same temperature before use. Rapid temperature fluctuations can also lead to the formation of air bubbles.

2. Sample preparation

Your sample should be completely dissolved. To extend the life of the column, run the sample through a filter with a pore size of 0.45 µm before loading the column. High viscosity samples require a buffer with a viscosity no greater than 1.5 times the sample.

In size exclusion chromatography, it is important to keep the concentration of your sample as high as possible. Accordingly, it is recommended to concentrate the sample down by centrifugation beforehand. However, how high you can keep the concentration depends on your biomolecules. As a general rule, the protein concentration should be below 70 mg/ml for proteins and below 5 mg/ml for high molecular weight polymers (>1000kD).

If possible, select a pre-packed column suitable for the sample volume to be desalted.
The table gives you an overview of which column is best suited for which sample volumes.

3. Column preparation and equilibration

a) Bring the column to your working temperature.

b) Rinse the system tubing with buffer. Remove the column inlet plug. Connect the column inlet to the system tubing without allowing air bubbles to enter the column.

c) Remove the end cap from the column outlet and run your buffer (for 1 ml column: min. 10 ml with flow rate max. 3 ml/min, for 5 ml column: min. 25 ml with flow rate max. 5 ml/min) to remove the storage ethanol and to equilibrate the column completely with your buffer.

4. Application and elution of the sample

a) Apply your sample (for 1 ml column: 0.05 - 0.3 ml, for 5 ml column: 0.1 - 1.5 ml). Monitor the column efflux with a UV, conductivity, fluorescence or other detection system. Note the optimal flow rate of 0.5 to 3 ml/min for 1 ml columns and 1 to 10 ml/min for 5 ml columns. Collect eluent fractions to recover the purified sample.

b) Rinse the column (for 1 ml column: min. 10 ml with flow rate max. 3 ml/min, for 5 ml column: min. 25 ml with flow rate max. 5 ml/min) before processing the next sample. Monitor the column effluent with a UV, conductivity, fluorescence or other detection system to ensure that the column is ready.

Warning H226

Full text of hazard- and precautionary statements see material safety data sheet section 2.2

Size exclusion chromatography columns from Carl Roth		
ROTI®Dex-25 Superfine FPLC 1 ml	**5 Pcs.**	**21C6.1**
ROTI®Dex-25 Superfine FPLC 1 ml	**100 Pcs.**	**21C6.2**
ROTI®Dex-25 Superfine FPLC 5 ml	**50 Pcs.**	**21C7.1**
ROTI®Dex-25 Superfine FPLC 5 ml	**25 Pcs.**	**21C7.2**
ROTI®Dex-25 Superfine FPLC 5 ml	**100 Pcs.**	**21C7.3**