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

ROTI®Dex columns for chromatography by centrifugation

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 Centrifugation columns are used for purification and desalting of small sample volumes (optimally 50 µl) in less than 5 minutes.

The column bed consists of ROTI®Dex-25 Medium (Order No. 21A5) or of ROTI®Dex-50 Medium (Order No. 21A6). It is a spherical, porous gel filtration medium consisting of dextran cross-linked with epichlorohydrin. The medium was swollen in deionized water.

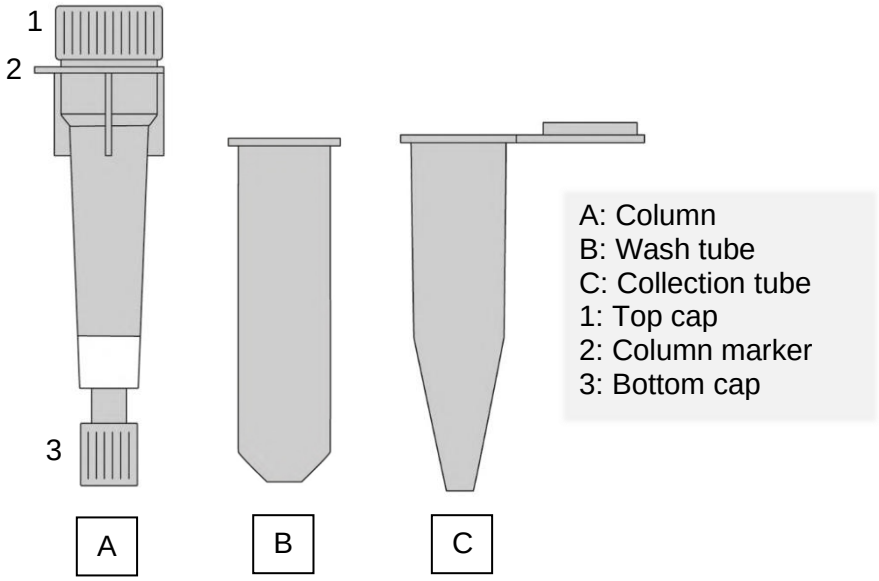
For further information on ROTI®Dex, please refer to our Technical Information Brochure.

Technical data of the ROTI®Dex Centrifugation columns:

Order No.	Matrix	Particle size (wet)	Gel bed volume	Sample volume	MWCO	Pack Qty.
21C4.1	ROTI®Dex 25 Medium	85 - 260 µm	0.5 ml	2 - 100 µl (50 µl optimal)	Proteins >5 kDa, Oligonucleotides >10 bp, Nanoparticles >2 nm	25 Pcs.
21C5.1	ROTI®Dex 50 Medium	100 - 300 µm	0.5 ml	2 - 100 µl (50 µl optimal)	Proteins >25 kDa, Oligonucleotides >20 bp, Nanoparticles >4 nm	25 Pcs.

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

Attention! The use of this product is intended for qualified personnel only. Suitability for use must be determined by the end user.
Read the centrifuge manufacturer's instructions and tare the samples properly before using the centrifuge.



1. Sample preparation

a) 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. For example, protein concentrations up to 70 mg/ml should not affect the separation when using normal aqueous buffers.

b) The sample should be completely dissolved.

2. Column preparation

a) Bring the column incl. equipment to your working temperature.

b) When working with several samples, we recommend labeling your column units accordingly. Label the wash(B) and collection(C) tubes on the outer wall. Please do not label the columns.

c) Remove the column(A) from the wash tube.

d) It is important for size exclusion chromatography that the complete gel bed volume is used. Due to transport or storage of the columns, it often happens that the gel bed is distributed in the column. Therefore, we recommend vortexing the column carefully until the gel bed has completely settled to the bottom. If the gel bed is also in the area of the top cap, vortex the column upside down as well.

e) Check whether the gel bed is free of air bubbles, otherwise vortex again.

f) Remove the top cap(1) and bend off the bottom cap(3).

g) Place the column back into the wash tube.

3. Remove storage buffer and equilibrate column

a) Place the column unit in your centrifuge. Align the column mark(2) so that it points outwards.

b) Tare the tubes and centrifuge at 1000 x g for 2 minutes.

c) Remove the column unit from the centrifuge and discard the wash tube including the contained storage buffer.

Optional:
Extra wash step for higher desalting efficiency: add 400 µl of deionized water to the column and repeat step 3(a) to (c).

or:

d) Equilibrate with buffer of choice: Unless you want your sample to elute in water, you can equilibrate the column at this point with your desired sample buffer. Add 400 µl of your buffer to the column and repeat step 3 a) to c). Repeat this procedure.

Note: Some proteins and nanoparticles may precipitate when eluted in low ionic strength water.

4. Application and elution of the sample

a) Up to 100 µl sample volume is possible. A sample volume of 50 µl is recommended.

b) Carefully apply your sample to the gel bed. Make sure that you apply the sample as centrally as possible without touching the gel bed.

c) Place your column into one of the collection tubes and place the column unit into your centrifuge. Again, make sure that the column mark and also the lid of the collection tube are facing outward.

d) Tare the tubes and centrifuge at 1000 x g for 2 minutes.

e) Remove the column unit from the centrifuge and discard the column. Your purified sample is in the collection tube.

Size exclusion chromatography columns from Carl Roth		
ROTI®Dex-25 Medium Spin	**25 Pcs.**	**21C4.1**
ROTI®Dex-50 Medium Spin	**25 Pcs.**	**21C5.1**