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

ROTI®Dex columns for gravity chromatography

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 Gravity columns are used for purification and desalting of sample volumes between 0.15 to 10 ml.

The column bed consists of ROTI®Dex-25 Medium (Order No. 21A5). 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.

In the following you will find our recommended protocol and other important instructions for use of the prepacked ROTI®Dex Gravity columns size XS to XXL. It also serves as an assistance for self-packed columns with our ROTI®Dex gel filtration media. Please also refer to the instructions for use for packing and storing the columns in our webshop.

This protocol is only a guide and should be adapted according to your specific needs.

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.

Sample preparation

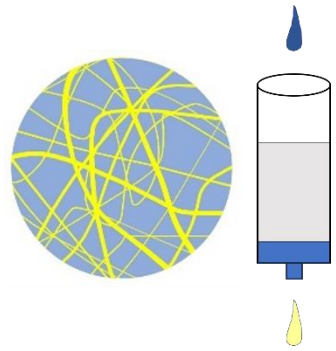
In size exclusion chromatography it is important to keep the concentration of your sample as high as possible. Accordingly, it is recommended to concentrate the sample down by centrifugation beforehand. However, how high you can keep the concentration depends on your biomolecules. For example, protein concentrations up to 70 mg/ml should not affect the separation when using normal aqueous buffers. The sample should be completely dissolved. Desalting generally results in dilution of the sample (except for spin protocols). Minimum dilution is achieved when the maximum desalting capacity of the column is used. Accordingly, use a large sample volume (≤ 30% of the total bed volume) to minimize sample dilution. Using a sample volume greater than 30% of the bed volume will result in less efficient desalting. Decrease the sample volume (< 30% of column volume) when the highest resolution is required for separation. If conductivity cannot be monitored and recovery of the fully desalted sample is the most important requirement, use a sample volume between 15% and 20% of the total bed volume.

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.

Order No.	Column size	Particle size (wet)	Gel bed volume	Sample volume	Elution volume	MWCO	Pack Qty.
21AX.1	XS	85 – 260 µm	1.78 ml	0.15 – 0.3 ml	0.35 ml	Proteins >5 kDa	50 Pcs.
21AY.1	S		2.75 ml	0,5 ml	1 ml	Oligonucleotides >10 bp	50 Pcs.
21C0.1	M		4.31 ml	1 ml	1.5 ml		50 Pcs.
21C1.1	L		10.37 ml	2.5 ml	3,5 ml		25 Pcs.
21C2.1	XL		17.2 ml	5 ml	7 ml	Nanoparticles >2 nm	10 Pcs.
21C3.1	XXL		34.21 ml	10 ml	14 ml		10 Pcs.

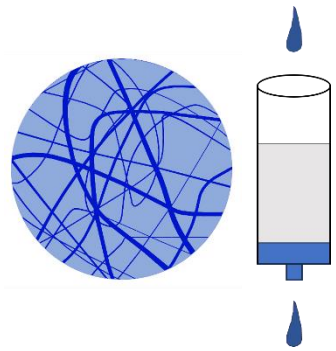
Tab. 1 Application note of ROTI®Dex columns

❖ The same buffer is used for the equilibration of the column as well as for the elution of the sample.



1. Column preparation

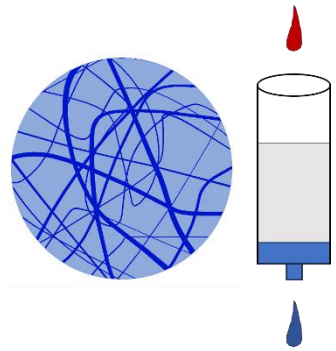
Bring the column to your working temperature and remove the top and then the bottom column cap. Storage buffer is eluted and desired buffer in which to transfer the sample is added.
The column matrix should never run completely dry.



2. Column equilibration

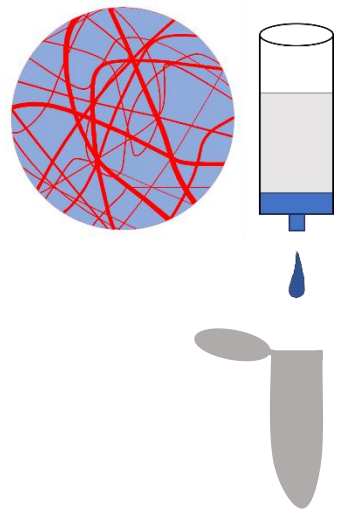
Apply the desired buffer several times (2 to 3 times) and elute to equilibrate column. Storage buffer should be completely removed if it does not correspond to the desired buffer.

❖ Take the respective **sample volumes** and **elution volumes** from Table 1.



3. Sample application

When the buffer has completely entered the gel bed, transfer the appropriate volume of sample for the respective column. Make sure that you apply the sample as centrally as possible and allow the sample to enter the gel bed completely.



4. Elution

Place a collection tube under your column and transfer the appropriate amount of elution buffer into the column to elute the biomolecules. Low molecular weight factors, such as salts, will remain in the matrix particles for the time being. Accordingly, it is important to clean and equilibrate the columns before the next use. You can find more information on this in our instructions for use on the subject of packing and storing ROTI®Dex columns.

Size exclusion chromatography columns from Carl Roth		
ROTI®Dex-25 Medium Grav XS	**50 Pcs.**	**21AX.1**
ROTI®Dex-25 Medium Grav S	**50 Pcs.**	**21AY.1**
ROTI®Dex-25 Medium Grav M	**50 Pcs.**	**21C0.1**
ROTI®Dex-25 Medium Grav L	**25 Pcs.**	**21C1.1**
ROTI®Dex-25 Medium Grav XL	**10 Pcs.**	**21C2.1**
ROTI®Dex-25 Medium Grav XXL	**10 Pcs.**	**21C3.1**