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

ROTI®Dex columns packing and storing

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

An efficiently packed column is essential for high resolution results. Below you will find our recommended protocol for packing and storing columns with our ROTI®Dex gel filtration media. Please also refer to the instructions for use of packed columns in the web store.

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

A column bed height of max. 10 cm is recommended for buffer exchange.

Step 1: Swelling of the gel filtration medium

ROTI®Dex is supplied as a dry powder and must be swollen before use.
Water, buffer or salt solutions are suitable as swelling solutions. ROTI®Dex particles shrink in alcohol solutions. If necessary, solutions should not contain more than 20% alcohol. (Filter all buffers through a 0.22 µm filter to prevent microbial growth).

1. Weigh out the appropriate amount of dry ROTI®Dex for the required bed volume of your column: 1 g ROTI®Dex-25 results in approx. 5 ml bed volume and 1 g ROTI®Dex-50 results in approx. 10 ml bed volume.

2. Add enough swelling solution (total volume of the column plus 30%) and allow the gel to swell for at least 3 hours at room temperature or 1 hour at 90 °C. Avoid using mechanical stirring aids (magnetic stirrers, spatulas, etc.).

3. After completion of swelling, the supernatant is decanted if the gel filtration is to be continued with a different buffer.

Step 2: Choose the right buffer

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.

Step 3: Column filling and packing

1. Before using the column, the column filter should be moistened without damaging or moving it. Use enough buffer or water so that the filter is completely saturated with liquid.

2. Prepare a suspension of buffer and the swollen ROTI®Dex particles that is liquid enough so that no air bubbles remain. A ratio of settled gel volume to buffer volume of 3:1 is optimal for this purpose.

3. Pour the entire suspension into the column, preferably in one go. Guiding the suspension along a glass rod or on the inner wall of the column can help to avoid air bubbles. Alternatively, a funnel can be used with the tip of the funnel touching the inner wall of the column. Care should be taken in principle to avoid trapping air bubbles.

4. A peristaltic pump can be used to pack the column. Ideally, the column should be packed with the highest possible pressure without deforming the beads. ROTI®Dex can be pressurized up to 3 bar. However, you can also let the buffer drip until the gel bed has settled evenly. Please make sure that you always refill buffer so that the gel bed does not run dry.

5. Allow another 2-3 column volumes of the buffer to be used for separation to pass through. This will stabilize and equilibrate the gel bed.

6. The gel bed should never run completely dry during the entire process and also during use. Please make sure that the gel bed is always in the buffer solution.

Step 4: Cleaning

1. Wash the column with two column volumes of 0.2 M NaOH or a solution of a non-ionic detergent. Then run 2 column volumes of water through the column.

2. Re-equilibrate the gel with 2-3 column volumes of buffer before the next experiment. If necessary, the gel can be removed from the column and sterilized by autoclaving at 120 °C, pH 7.

Step 5: Storage

Store used gel at 2-8 °C in 20% ethanol or in a solution of a microbial growth inhibitor such as 0.002% hibitan/chlorhexidine or 0.02% sodium azide.
Do not freeze. Before the next use, the gel should be adjusted to the ambient temperature.

Gel filtration medium from Carl Roth		
ROTI®Dex-25 Medium	**10 g**	**21A5.1**
ROTI®Dex-25 Medium	**50 g**	**21A5.2**
ROTI®Dex-25 Medium	**100 g**	**21A5.3**
ROTI®Dex-25 Medium	**250 g**	**21A5.4**
ROTI®Dex-25 Medium	**500 g**	**21A5.5**
ROTI®Dex-50 Medium	**10 g**	**21A6.1**
ROTI®Dex-50 Medium	**50 g**	**21A6.2**
ROTI®Dex-50 Medium	**100 g**	**21A6.3**
ROTI®Dex-50 Medium	**250 g**	**21A6.4**
ROTI®Dex-50 Medium	**500 g**	**21A6.5**

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