TECHNICAL NOTES SEI

Biochemicals

Electrophoresis Bioseparation

Life Sciences Specials

CentriPure Gelfiltration Columns

CentriPure Gelfiltration Columns are designed for fast and efficient removal of small molecules such as salts, ammonia, dyes, biotin, haptens etc. from antibodies, enzymes and other proteins. The columns are sterile packed, pre-swollen, and ready to use.

Zetadex Gel Matrix

The gel matrix of CentriPure Gelfitration Columns is Zetadex, a beaded composite material developed by emp BIOTECH composed partially of polymerized dextran. Zetadex exhibits high selectivity, high resolution and chemical stability. It has been proven to be superior for desalting, buffer exchange and removal of small molecular impurities from protein solutions.

Separation with CentriPure

Molecules purified with Zetadex are separated according to size. Smaller molecules pass significantly slower through the column than larger molecules allowing effective separation of small molecular impurities from proteins. Buffer and pH effects on resolution are minimal. Therefore equilibration and elution buffer for CentriPure Flow Gravity Columns like CentriPure P2, can be chosen optimal for the specific application.

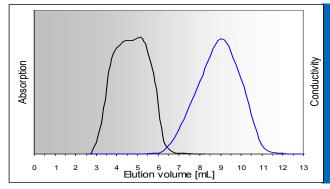
- Fast and easy handling
- High yield and high purity
- Minimal effect of buffer and pH on resolution
- Purified biomolecules are directly suited for downstream applications, like SDS PAGE, mass spectrometry, X-ray crystallisation

CentriPure Gelfiltration Columns

Rapid purification of proteins

I. CentriPure Flow Gravity Gelfiltration Columns

Gel filtration with CentriPure columns is done without pre-swelling of the matrix or cumbersome column packing. An easy four step protocol allows the fast purification of proteins with low dilution from sample volumes of 200 μ l up to 10 ml. The size exclusion cutoff for Zetadex 25 is 10 kD for proteins.



Highly effective desalting of protein solution: Elution profile of CentriPure column P2. Applied was an ovalbumin solution in 0.8 M NaCl and elution was done with water.

II. CentriPure Mini Spin Gelfiltration Columns

The columns are available with two size exclusion cut-offs: Z-50 Spin Columns for purification of proteins \geq 25 kDa and Z-25 Spin Columns for proteins \geq 5 kDa. The spin columns are hydrated in deionized water, Phosphate Buffered Saline (PBS, pH 7) or 1 mM TRIS, pH 6. Some proteins may precipitate in pure water with low ionic strength and therefore the use of PBS or TRIS hydrated columns is necessary. Sample volumes between 10 and 100 μ l can be processed. Optimal purification and recovery is obtained with a sample volume of 50 μ l. The easy and fast centrifugation protocol results in minimal dilution of the protein sample.

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Gel filtration protocol with Flow Gravity CentriPure columns



Column Preparation

Remove caps from top and bottom. Allow excess column fluid to drain into a waste reservoir



Column Equilibration

Equilibrate the different column types with a buffer according to your specific application. P2 and P5: 5 ml, P10: 15 ml, P25: 25 ml, P50: 40 ml, P100: 80 ml



Sample Application

Transfer sample to column, allow it to enter gel bed completely.



Elution

Transfer appropriate elution buffer volume (same buffer as equilibration buffer) to column, elute sample into a collection tube.

Ordering information

| Product (sample / elution volume) | Size (columns) | Cat. No. |
|---|-------------------|----------|
| CentriPure P2 Columns | 2 | 42100.01 |
| (up to 200 μ l / 200 – 300 μ l) | 50 | 42101.01 |
| CentriPure P5 Columns | 2 | 42102.01 |
| (up to 0.5 ml / 1 ml) | 50 | 42103.01 |
| CentriPure P10 Columns | 2 | 42104.01 |
| (up to 1 ml / 1.2 – 1.5 ml) | 50 | 42105.01 |
| CentriPure P25 Columns | 2 | 42106.01 |
| (up to 2.5 ml / 2.7 – 3.5 ml) | 25 | 42107.01 |
| CentriPure P50 Columns | 2 | 42108.01 |
| (up to 5 ml / 6 – 8 ml) | 10 | 42109.01 |
| CentriPure P100 Columns | 2 | 42110.01 |
| (up to 10 ml / 12 – 15 ml) | 10 | 42111.01 |



Rapid purification of proteins

Five minutes protocol for CentriPure Mini Spin Gelfiltration columns

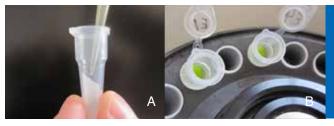
Desalting, buffer exchange or removal of labels from protein samples is done in a five minute protocol using standard lab bench centrifuges.

1. Column preparation

- Tap gently or briefly vortex to resuspend gel and remove air bubbles
- Remove bottom cap and then remove top cap
- Place the column into a wash tube
- \cdot Centrifuge at 1000 x g for 2 minutes
- Discard wash tube and eluted storage buffer

2. Sample application and elution

- · Carefully apply sample directly to center of gel bed (A)
- Place column into a collection tube, centrifuge at 1000 x g for 2 minutes to elute the sample (B)



Ordering Information

| Product | Size (columns) | Cat. No. |
|---|-------------------|----------------------------------|
| CentriPure Mini Spin Columns Desalt Z-50 | 4 25 100 | 42113.01 42114.01 42115.01 |
| CentriPure Mini Spin Columns TRIS Z-50 | 4 25 100 | 42124.01 42125.01 42126.01 |
| CentriPure Mini Spin Columns PBS Z-50 | 4 25 100 | 42127.01 42128.01 42129.01 |
| CentriPure Mini Spin Columns Desalt Z-25 | 4 25 100 | 42130.01 42131.01 42132.01 |
| CentriPure MINI Spin Columns TRIS Z-25 | 4 25 100 | 42133.01 42134.01 42135.01 |
| CentriPure MINI Spin Columns PBS Z-25 | 4 25 100 | 42136.01 42137.01 42138.01 |



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