AppliCations

No.6

Size-Exclusion Chromatography for purification of biomolecules

Size-exclusion chromatography (SEC) is a popular method to separate biomolecules based on their size. Primarily, it is applied to the separation of biopolymers such as proteins and nucleic acids, i.e. water-soluble polymers. This system is also called gel filtration, typically with beads of dextran or agarose serving as gel matrix. Smaller molecules pass significantly slower through the column than larger molecules. Not to be mixed up with gel electrophoresis, there are big differences in terms of the separation principle. SEC does not require electric current and the sieving effect will not separate small molecules first.

AppliChem

Keywords

Gel filtration matrix

cross-linked dextran beads

Desalting

Buffer exchange

Nucleic acid/ protein purification

Dye terminator removal

It is indeed correct that smaller molecules pass more slowly through the matrix than larger molecules. This is due to the longer path the smaller molecules must travel. The longer path arises from the pores of the beads. The smaller molecules can enter the pores and go inside the beads. Entering the pores creates a longer path for the smaller molecules. There is a relationship between the delay this causes and the molecular size. The larger molecules can not enter the pores and therefore have a shorter path. The larger molecules therefore travel faster through the column than smaller molecules.

Advantages of SEC/Gel Filtration

Some important features of the technique have to be mentioned. First, that the separation of the large molecules from the small ones is very effective and is performed under mild conditions does mean that biomolecules retain their biological activity. The volume of the eluate may be kept small and therefore the technique is suited for concentrated samples.

The Matrix

AppliChem's size-exclusion chromatography columns are packed with AppliXchange-G25 M, a beaded composite material composed partially of polymerized dextran. It exhibits high selectivity, high resolution and chemical stability. Molecules purified with AppliXchange-G25 M are separated according to size. The chemical interaction of gel matrix and molecules to be separated is negligible. Little or no absorption or binding takes place. Buffer and pH effects on resolution are kept minimal. Therefore, buffer conditions, pH value and temperature during the filtration process may be adapted according to the needs of the molecules but not the column material. Essential co-factors or ions necessary for the function or stability of proteins may be included in the eluent as well, since they largely don't interfere with the separation. The size exclusion cut-off for AppliXchange-G25 M is set at 10 kD for proteins and 10 bp for nucleic acids. Purified biomolecules are not significantly diluted when processed using AppliXchange-G25 M.

Experience shows that columns may be used repeatedly, up to four times. It is essential to include an additional washing step of the columns with elution buffer after separation to remove residual molecules.

The number "25" correlates to the "water regain" of the matrix. A grade "25" means that the dry matrix will take up 2.5 times its weight of water (1 g AppliXchange-G25 M absorbs 2.5 g water). This is a water regain of 2.5. Likewise, a grade of "50" gives a water regain of 5 grams (1 g AppliXchange-50 plus 5.0 g water.) The ability to absorb water is dependent on the

extent of cross-linking. Tighter cross-linking limits the ability of the beads to swell and as a consequence the water regain is lower. Less cross-linking gives a more flexible bead and allows additional swelling. The extent of cross-linking has been optimized for a particular water regain. The water regain/extent of cross-linking affects the separation properties of the gels. Larger water regain/less cross-linking results in larger pores, which in turn retains larger molecules. Less water regain/more cross-linking results in smaller pores, which in turn retains only the smallest molecules. The separation efficiency is ultimately dependent on the AppliXchange grade, flow rate, column diameter, column length, buffer eluent and size difference of the sample components.

The superfine, fine, medium and coarse designations describe the average size of a fraction of bead diameters. Superfine refers to 20–50 µm diameters, fine 20–80 µm, medium 50–150 µm, coarse 150–200 µm, where 80 % of the fraction is within the given diameter range. The average diameter distribution affects the flow rate through the medium. Typically, plates use superfine, spin columns use superfine or fine, smaller gravity columns use medium and larger columns use medium or coarse.

DextraSEC NA10 and DextraSEC PRO10

"NA" stands for nucleic acids, "PRO" for protein. The number "10" correlates to the volume of the sample to be processed, i.e. columns are for the purification of 1.0 ml nucleic acid / protein sample. Typically, oligonucleotides are purified from salts, ammonia, and other small molecules. For example, after a labeling reaction with biotin or a dye, the excess biotin or dye is removed by using this column. The oligonucleotide size should be 10 bp or more to obtain satisfactory recovery. Comparably, proteins may be desalted or purified from dye. The protein size should be 10 kD or more to obtain satisfactory recovery.

The DextraSEC products are available both in column or plate (96- or 384-well plates) formats.

Stability of the Matrix

The issue is one of unwanted microbiological growth and not a physical problem associated with the AppliXchange. The hydrated matrix may be stored at room temperature indefinitely when hydrated in the presence of a preservative, such as sodium azide, thimerosal, or other commercially available biocides. We use Proclin®300.

If the AppliXchange matrix is hydrated with water, or worse, in PBS without a preservative, significant and visible bacterial or fungal growth will eventually be observed. When this will occur depends on the conditions in which the AppliXchange was hydrated, the cleanliness of the containers used, the purity of the water or buffers, etc. Since these are all uncontrolled conditions, we recommend to store rehydrated AppliXchange-G25 M (without preservative) at 2 to 8°C for no longer than one week.

We have hydrated AppliXchange-G25 M using very clean glassware, highly purified and sterile water, and then stored at 6°C without observing any bio-growth for over 6 weeks. We have also stored the same for up to 2 to 3 days (weekend) at room temperature without problems. Please note that phosphates act like fertilizers. If desired, PBS is a fine hydration and/or elution buffer. But the columns should not be stored at room temperature in PBS without preservatives!

Overload

"Load" can refer to either the sample volume or concentration. Too much of either will overload the column and this incorrect use of the product can be corrected by simply reducing the load. Do NOT add more volume than recommended and do NOT add an "unreasonably" high concentration of impurities and/or the molecules to be purified. This will lead to a less than satisfactory purification. If there is an exact, definable application, then we may provide a maximum allowed concentration and sample volume on request. Without this, you may have to determine your own parameters for your particular application.

Instructions for Hydration (Bulk)

AppliXchange-G25 M is provided as a dry powder. Before use, it must be hydrated. Do not stir the hydrating AppliXchange excessively during hydration, as this will damage the beads. Do not use magnetic stirrers.

AppliXchange-G25 M medium will swell to a volume of approximately 5 ml for every one gram of dry AppliXchange-G25 M. As a general rule, use 6.5 to 10 ml hydration buffer or water for every gram AppliXchange-G25 M that is to be hydrated.

Choose the bed volume required for your application, determine the number of grams of AppliXchange-G25 M required and then determine the volume of hydration buffer or water required.

Hydrate AppliXchange-G25 M at room temperature for 3 hours or at 90°C for 1 hour. Hydrated AppliXchange-G25 M may be sterilized at neutral pH by autoclaving for 30 minutes at 120°C. For best results, the hydrated slurry should be degassed before use.

For re-use, the hydrated gel can be washed with 2 column-volumes of 0.2 M NaOH, rinsed with 2 column-volumes water, and re-equilibrated with 2–3 column volumes of buffer.

For storage, antimicrobial agents should be added to the suspension to prevent contamination (0.001 % phenyl mercuric salts, 0.005 % thimerosal, 0.05 % chlorobutanol, 0.002 % chlorhexine, 0.02 % sodium azide, or 20 % ethanol are acceptable). Hydrated AppliXchange-G25 may be stored at room temperature (preservative included!) or at +2 to +4°C. Do not freeze! Allow the gel to equilibrate to room temperature before use.

Instructions for Column Handling

1. Column Preparation

Remove the red cap from the top and then the bottom cap of the DextraSEC NA10 Column. Allow excess column fluid to drain (via gravity) into a suitable waste reservoir.

2. Column Equilibration

Choose a buffer for your specific application and use this same buffer for both equilibration and elution steps. To equilibrate the column, allow the equilibration buffer to enter the gel bed completely and continue elution until approximately 15 ml of buffer has been eluted.

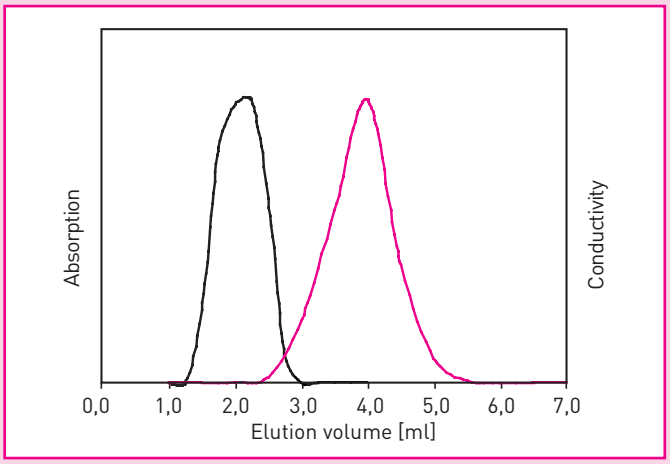
3. Sample Application

Transfer up to 1 ml of your sample to the DextraSEC NA10 Column. Allow the sample to enter the gel bed completely. If the sample volume is less than 1 ml, add enough equilibration buffer so that the combined volume equals 1 ml before applying it to the column.

4. Elution

Place a tube for sample collection under the DextraSEC NA10 Column. Transfer 1.5 ml of elution buffer to the column and elute the cleaned sample.

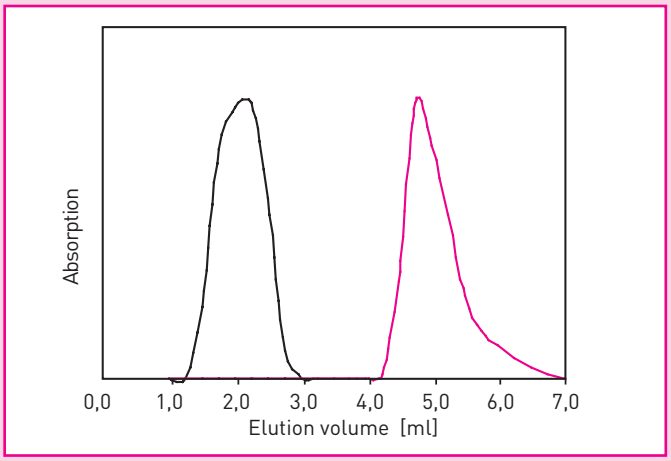
Protein Purification



Protein Desalting from 0.8 M NaCl

Protein (280 nm): black line NaCl (µS): pink line

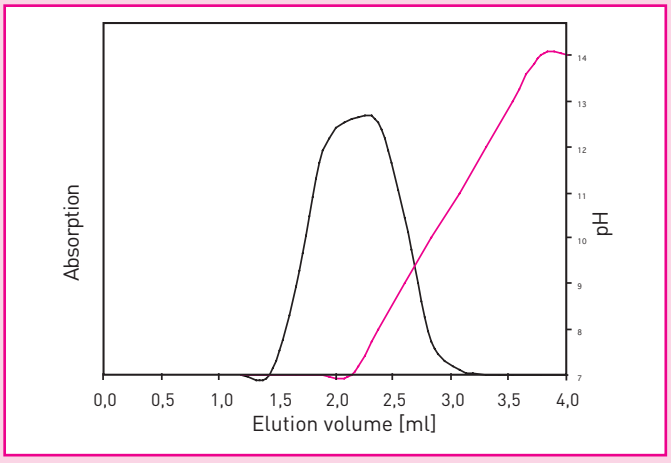
1 mg IgG anti-Rabbit in 1 ml 0.8 M sodium chloride. Elution with pure water.



Removal of FITC from IgG

IgG (280 nm): black line FITC (490 nm): pink line

1 mg IgG anti-Rabbit and 0.1 µmol FITC in 1 ml DMSO/$NaHCO_3$. Removal of excess FITC after coupling reaction. Elution with PBS.

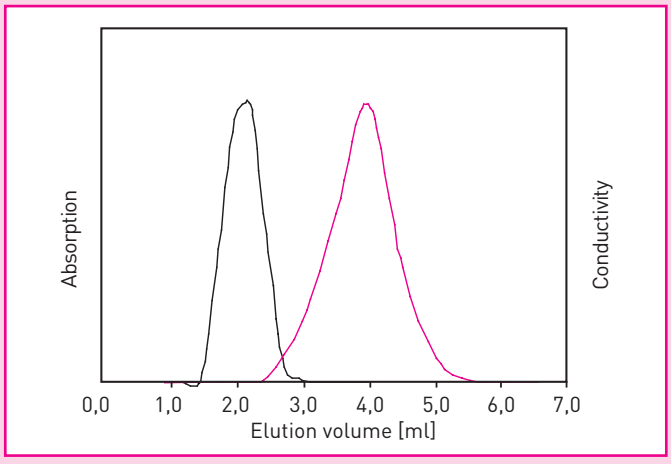


Removal of ammonia (33 % NH_3) from Dextran Blue

Dextran Blue (620 nm): black line NH_3 (pH): pink line

0.5 mg Dextran Blue (M_r 2,000,000) in 1 ml ammonia (33 %). Elution with pure water.

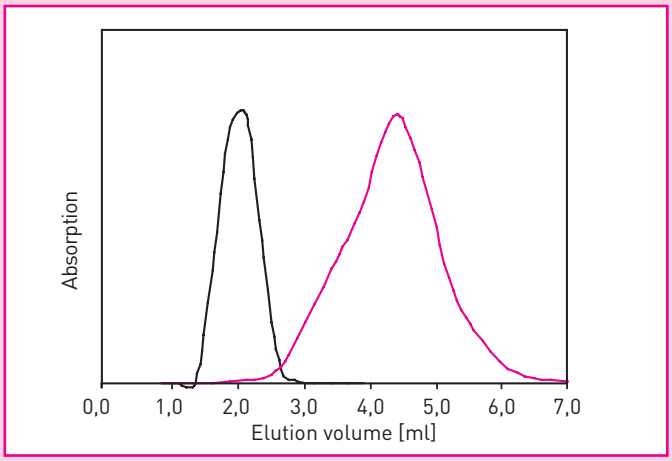
Oligonucleotide Purification



Oligo Desalting from 0.8 M NaCl

Oligo (260 nm): black line NaCl (µS): pink line

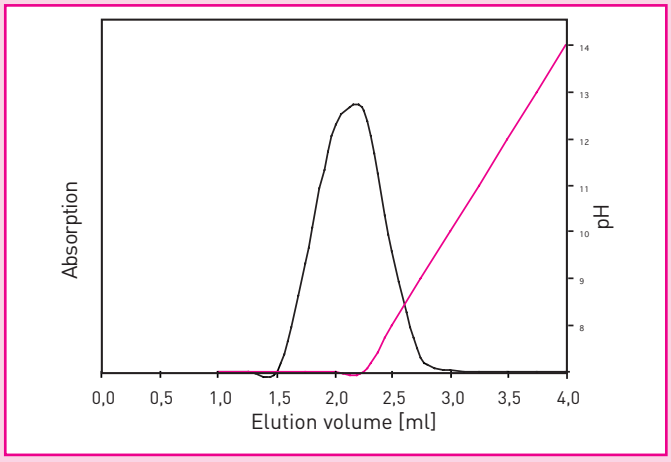
1 mg oligonucleotide (18-mer) in 1 ml 0.8 M sodium chloride.



Removal of Rhodamine from Oligonucleotide Labelling

Oligo (260 nm): black line TAMRA (550 nm): pink line

1 mg oligonucleotide (18-mer) and 0.5 µmol TAMRA in 1 ml DMSO/$NaHCO_3$. Removal of excess TAMRA after coupling reaction.



Removal of ammonia (33 % NH_3) from Nucleic acid

Oligo (260 nm): black line NH_3 (pH): pink line

1 mg oligonucleotide (18-mer) in 1 ml ammonia (33 %) after cleavage from solid support and protection group removal.

A95,E

Typical Analytical Data of AppliXchange-G25 M (Product No. A8507)

CAS Number	9041-35-4
HS No.	39139000990
Appearance	white solid
Fractionation range (globular proteins) – M_r	1000 – 5000
Fractionation range (dextrans) – M_r	100 – 5000
Bead structure	Cross-linked dextran composite
Bead size (Dry Particle Size)	50 – 150 µm
Bead size (Wet)	85 – 260 µm
Bed Volume in Water	4 – 6 ml/g
Maximum operating pressure	Obeys Darcy's Law
Chemical stability	All commonly used buffers, including: 0.2 M NaOH; 0.2 M HCl; 1 M Acetic acid; 8 M Urea; 6 M Guanidine HCl; 1 % SDS, 24 % Ethanol; 30 % Propanol; 30 % Acetonitrile.
pH stability	2.0 to 13.0,
Autoclavable	at 121°C, pH 7 for 30 minutes
Storage	ambient (Do not freeze!)

High Performance Results:

- Over 90 % recovery
- *Easy-to-use*
- High chemical stability
- High resolution

Description	Product No.	Quantity	Sample Volume
DextraSEC NA10	A8870,0050	50 Columns	0.5 – 1 ml
DextraSEC PRO10	A8822,0050	50 Columns	0.5 – 1 ml
DextraSEC 96W	A8595,0296	2 Plates	max. 15 µl/well*
DextraSEC 96W	A8595,2596	25 Plates	max. 15 µl/well*
DextraSEC NA2	A8590,0050	50 Columns	0.15 – 0.25 ml
DextraSEC PRO2	A8710,0050	50 Columns	0.15 – 0.25 ml

coming soon: DextraSEC 384W 384-Well Gel Filtration Plates for sample volumes of max. 10 µl

*Main application: dye terminator removal in automated DNA sequencing procedures

AppliChem

AppliChem GmbH | Ottoweg 4 | D-64291 Darmstadt | Phone +49 6151 93 57-0 | Fax +49 6151 93 57-11 |
eMail service@applichem.com | Internet www.applichem.com

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4t Matthes + Traut · Darmstadt