Protocol

TD-P Revision 3.0

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Gel Filtration Chromatography Protocol

Utilizing Plain and Cross-linked Agarose Beads

Introduction

Gel filtration is a technique used to separate proteins by differences in their molecular size. During the filtration process, molecules are separated into two different phases: the stationary phase (a matrix composed of porous beads) and the mobile phase. Molecules of different sizes, with different number of charges and distribution of charge groups, will enter the pores in the matrix (agarose) or become part of the mobile phase, with smaller molecules typically eluting more slowly than larger ones. These proteins are then removed from the agarose using elution buffers designed to elute proteins of a specific molecular weight (Figure 1). Here, we include instructions and tips for packing of a column with agarose beads (plain and cross-linked) and activating beads for biomolecule purification or immobilization. Beads can be used in column or batch format.

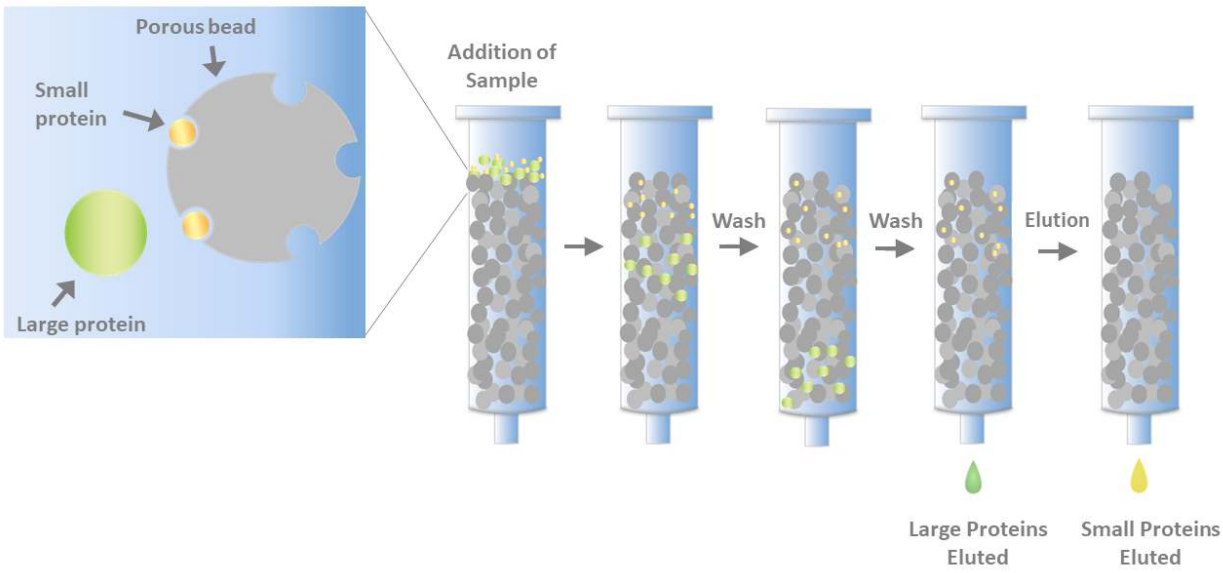

Figure 1. Overview of size-exclusion chromatography (also called gel filtration). In this assay, proteins are separated according to size.

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Materials

- Cross-linked (GoldBio Catalog # A-171, A-172, A-191, A-192, or A-193) or plain (GoldBio Catalog # A-173, A-174, A-181, A-182, A-183, A-184, or A-185) agarose beads in varying percentage.
- Equilibration buffer
- Elution buffer
- dH_2O

Method

Column packing and equilibration

1. Manually shake the bottle to obtain a homogenous suspension of Plain or Cross-linked Agarose Beads and preservative.

   Note: It is recommended to degas all the solutions before adding them to the column to avoid formation of bubbles.

2. Place a funnel in the head of the column and slowly run suspension down the walls of the column.

   Note: It is advisable to add the suspension slowly to avoid bubbles. The product may also be degassed before it is added to the column.

3. Repeat previous steps until the desired column height is obtained.

4. Insert the adapter gently in the column head until it begins to displace the liquid. Ensure air is not trapped under the frit.

   Note: If any air bubbles remain, tap the column to remove them. If tapping does not remove them, centrifuge the column at a low RPM.

5. Connect the pump to the column and ensure the column height remains the same as the flow of dH_2O passing through.

6. When a constant height has been obtained, maintain the flow with the addition of 5 column volumes of dH_2O to completely eliminate the preservative.

7. Equilibrate the column with 2-5 column volumes of equilibration buffer.

Note: Addition of at least 0.2M of sodium chloride (NaCl) to the equilibration buffer is recommended to avoid ionic interactions.

Sample addition

1. Add the sample. The sample volume should be approximately 2-5% of the entire volume of the column.

2. Elute with 1 column volume of elution buffer.

Note: Before applying a new sample, the column must be re-equilibrated with equilibration buffer until the optical density (O.D.) at 280 reaches the baseline level and remains stable.

Regeneration

For regeneration and later reuse of agarose beads, wash the column with 3 volumes before re-equilibrating with a new buffer.

Note: If poor resolution or strange pressures are observed, insert a washing step before proceeding to the re-equilibration step. This washing step can be done at a high ionic strength (which eliminated precipitated or nonspecifically bound proteins) or with a non-ionic detergent.

Note: Keep the column/agarose beads in an appropriate preservative between uses.

Table 1. Stability

Product	Studies	Characteristics
Plain Agarose Beads	Thermal Stability	Not autoclavable
	Chemical Stability	- Stable in all solutions commonly used in Gel Filtration, including 8M urea and 6M guanidine hydrochloride. - Oxidizing agents are not advisable. - Stable in acid (pH 4.0) and basic (pH 9.0) solutions. - Resistant to biological degradation.
	Physical Stability	- Negligible volume variation due to changes in pH or ionic strength.

Product	Studies	Characteristics
Cross-linked Agarose Beads	Thermal Stability	- Autoclavable for 30 minutes at 121°C, at pH 7.
	Chemical Stability	- Stable to all solutions commonly used in Gel Filtration including 8M urea and 6M guanidine hydrochloride. - Stable in organic solvents such as ethanol, DMF, acetone, DMS, chloroform, dichloromethane, dichloroethane, pyridine, triethyl phosphate and acetonitrile. - Oxidizing solutions should be avoided Stable in strong acidic (pH 2.0) and strong basic (pH 13.0) solutions. - Dissociating agents and chaotropic salts (urea, guanidine, KSCN, DMS or similar reagents) can be used. - Resistant to biological degradation.
	Physical Stability	- Negligible volume variation due to changes in pH or ionic strength.

Tips

Depending on the purpose of your gel filtration, it may or may not be important to obtain a good resolution between peaks. If the objective is group separation (to separate two major components in a solution by size range), achieving a good resolution in all peaks is not as important. However, good peak resolution is vital if the goal is to separate different proteins in a sample according to molecular size (to determine molecular weight or isolate one or more proteins). This is called high resolution fractionation.

The most important factors affecting resolution generally are sample volume and column size. Sample volumes used when separating by size range (group separation), are larger than volumes used in high resolution fractionation (~15 times larger). To separate proteins by molecular size and obtain high peak resolution, sample volumes are typically less than 2% of the column volume, however, the volume can range from 1 to 5% of column volume. This quantity changes depending on the peak resolution of interest.

Here are some other recommendations for good resolution:

- Other aspects that may influence separation are percentage of agarose, molecular pore size, column packaging, and flow rate.
- When sample viscosity is too high, consider working at higher temperatures.
- While the buffer composition does not directly affect resolution, it can affect the protein stability and activity. Ionic strength, pH, and denaturing agents can produce conformational changes or protein dissociation. Choose a buffer that keeps pH constant and avoids nonspecific interactions.

Note: It is advisable to degas the buffers and prepare them in high-quality water prior to use.

- If detergents or denaturing agents are needed, they should be present in both the running buffer and sample buffer.

  Note: If detergent concentration is high then pressure flow is higher and flow reduction is necessary.

- Remove hydrophobic proteins or lipoproteins by washing the column with 4 column volumes of 1M NaOH, then wash the column with 4 column volumes of distilled water.

Analytical Method

(For maximum resolution)

- Beads as small as possible
- Column as large as possible
- Optimum gel pore size
- Small sample loading

Preparative Method

(For maximum throughput)

- Beads as large as possible
- Column as short and wide as possible
- Optimum gel pore size
- Largest permissible sample loading
- Fastest flow rate (highest permissible pressure)

Associated Products

- 4% Agarose Beads, Fine (GoldBio Catalog # A-173)
- 6% Agarose Beads, Fine (GoldBio Catalog # A-174)
- 2% Agarose Beads, Standard (GoldBio Catalog # A-181)
- 4% Agarose Beads, Standard (GoldBio Catalog # A-182)
- 6% Agarose Beads, Standard (GoldBio Catalog # A-183)
- 8% Agarose Beads, Standard (GoldBio Catalog # A-184)
- 10% Agarose Beads, Standard (GoldBio Catalog # A-185)
- 4% Agarose Beads, Fine, Cross-linked (GoldBio Catalog # A-171)
- 6% Agarose Beads, Fine, Cross-linked (GoldBio Catalog # A-172)
- 2% Agarose Beads, Standard, Cross-linked (GoldBio Catalog # A-191)
- 4% Agarose Beads, Standard, Cross-linked (GoldBio Catalog # A-192)

- 6% Agarose Beads, Standard, Cross-linked (GoldBio Catalog # A-193)

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