Technical Bulletin

Gel Filtration Markers Kit for Protein Molecular Weights 6,500-66,000 Da

MWGF70

Product Description

Gel filtration chromatography is an established method to determine the size and molecular mass of proteins. Fractionation is based on the diffusion of molecules into the pores of the resin. Larger proteins do not enter the pores of the resin as readily, but instead pass through the fluid volume of the column faster than smaller proteins. These protein molecules elute from the column in order of decreasing molecular mass.

The molecular mass determination of unknown proteins is made by comparing the ratio of V_e/V_o for the protein in question to the V_e/V_o of protein standards of known molecular mass, where:

- V_e is the elution volume
- V_o is the void volume

The V_o of a given column is based on the volume of effluent required for the elution of a large molecule such as Blue Dextran (molecular mass of ~2,000 kDa, Cat. No. D4772). Plotting the logarithms of the known molecular masses of protein standards versus their respective V_e/V_o values produces a linear calibration curve (such as shown in **Figure 1**).

V_e/V_o is essentially independent of column size and protein concentration. However, V_e/V_o may be dependent on temperature for some proteins. Unreliable molecular masses may be obtained if the protein:

- Forms a complex with the gel
- Contains a large amount of carbohydrate
- Aggregates to larger complexes
- Or dissociates into subunits under the conditions used.[1]

The molecular mass of an impure protein may be determined using this procedure if a specific detection test is available for the protein.

The procedure for determining molecular masses using gel filtration chromatography, as outlined in this bulletin, is a modification of published methods.[1,2] The protein standards in this kit may be suitable for use in other chromatographic systems such as HPLC, although some buffer systems seem to alter the elution volumes of albumin (Cat. No. A8531) and carbonic anhydrase (Cat. No. C7025). The proteins in the MWGF70 Kit have a range of molecular masses from 6.5 kDa to 66 kDa.

Several theses[4-5] and dissertations[6-13] cite use of product MWGF70 in their protocols.

Reagent

- Aprotinin from bovine lung, Cat. No. A3886: 15 mg/vial. Approximate Molecular Mass: ~6.5 kDa
- Cytochrome c from horse heart, Cat. No. C7150, 10 mg/vial. Approximate Molecular Mass: ~12.4 kDa
- Carbonic Anhydrase from bovine erythrocytes, Cat. No. C7025, 15 mg/vial. Approximate Molecular Mass: ~29 kDa
- Albumin, Bovine Serum, Cat. No. A8531, 50 mg/vial (contains ~0.3% dithiothreitol). Approximate Molecular Mass: ~66 kDa
- Blue Dextran, Cat. No. D4772, 50 mg/vial. Approximate Molecular Mass: ~2,000 kDa

Storage/Stability

Store the MWGF70 product at -20 °C.

Procedure

Use of MWGF70 for Gel Filtration Chromatography

1. Buffer and Resin:
   - It is recommended to use 50 mM Tris-HCl, pH 7.5, with 100 mM KCl as the equilibration buffer, with a 90 cm × 1.6 cm Sephadex® G-75-50 (Cat. No. G7550) column at 2-8 °C.
   - For information on resin preparation, column packing, and equilibration, contact Technical Service.
2. Void Volume (V_o) Determination:
   - Dissolve the Blue Dextran in equilibration buffer containing 5% glycerol at a concentration of 2 mg/mL.
   - This concentration of Blue Dextran will give an A280 of ~1.0 in the peak fraction.
   - Glycerol is added to increase the density of the solution, but its use is optional.
   - The recommended sample volume is less than 2% of the total gel bed volume.
   - Carefully apply the Blue Dextran sample to the column (avoid disturbing the gel bed surface) to determine V_o and to check the column packing.
   - Immediately after applying the sample, begin collecting fractions of 0.5-1.5% of the total gel bed volume. The flow rate should be ~7% of the column volume per hour.
   - Skewing of the Blue Dextran band represents a fault in the column, although some tailing is normal.
   - The leading peak indicates the void volume.
   - Determine spectrophotometrically the elution volume for Blue Dextran (V_o for the column) at 280 nm or 610 nm by measuring the volume of effluent collected from the point of sample application to the center of the effluent peak.
   - **Note**: Mixing Blue Dextran with kit standards or sample proteins is **not** recommended, since many proteins bind to Blue Dextran.
   - Prepare the protein standards and Blue Dextran fresh. Occasionally some aggregated protein may appear at the void volume.
3. Elution Volume (V_e) Determination for Protein Standards:
   - Dissolve individual protein standards in equilibration buffer containing 5% glycerol (see **Table 1**).
   - If, upon reconstitution, any of the protein solutions contain insoluble material, then filter the protein solution through a 0.45 µm or 0.2 µm filter. The loss of protein from this filtration is negligible.
   - For a 90 cm × 1.6 cm column, the application of 2.0 mL of individual samples at the recommended concentration (**Table 1**) gives an A_{280} of ~1 in the peak fraction.

Table 1: Recommended Protein Concentrations

Protein	**Recommended concentration**
Aprotinin	3 mg/mL
Cytochrome c	2 mg/mL
Carbonic anhydrase	2 mg/mL
Bovine Serum Albumin	5 mg/mL

   - The following pairs of proteins may be mixed and run together on the columns:
     - Albumin and Cytochrome c
     - Carbonic anhydrase and Aprotinin
   - Apply the protein standards to the column, using the same sample volume and flow rate as used for the Blue Dextran sample.
   - The elution of the standard proteins may be followed by absorbance readings at 280 nm.
   - Determine the V_e for the protein standards by measuring the volume of effluent collected from the point of sample application to the center of the effluent peak.

4. Standard Curve: Plot molecular mass vs. V_e/V_o for each respective protein standard on semi-log paper (see **Figure 1**).
5. Elution Volume (V_e) Determination for an Unknown Protein:
   - Apply the unknown sample to the column at an appropriate concentration using the same sample volume, fraction size, and flow rate as used for the Blue Dextran and the protein standards.
   - Determine the V_e of the unknown using the same methods applied to the standards.
   - Calculate the V_e/V_o for the unknown and determine its molecular mass from the standard curve.

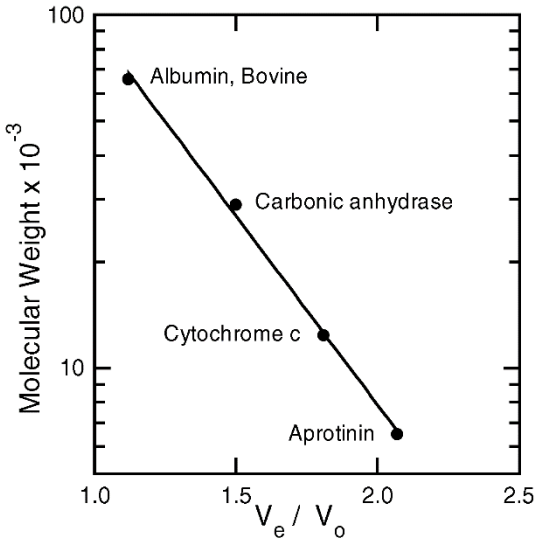

Figure 1. Typical calibration curve obtained with proteins from the MWGF70 Kit run on Sephadex® G-75-50.

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