SIGMA-ALDRICH®
sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Gel Filtration Molecular Weight Markers
Kit for Molecular Weights 12,000–200,000 Da

Catalog Number **MWGF200**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Gel filtration chromatography is an established method for determining 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 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 (V_e is the elution volume and 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, Catalog Number 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.

V_e/V_o is essentially independent of column size and protein concentration, but may be temperature dependent 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 including HPLC, although some buffer systems seem to alter the elution volumes of albumin (Catalog Number A8531) and carbonic anhydrase (Catalog Number C7025). The proteins in the MWGF200 Kit have a range of molecular masses from 12.4–200 kDa.

Reagents

	Approximate Molecular Mass
• Cytochrome c From horse heart, Catalog Number C7150, 10 mg/vial	12.4 kDa
• Carbonic Anhydrase from bovine erythrocytes, Catalog Number C7025, 15 mg/vial	29 kDa
• Albumin, bovine serum, Catalog Number A8531, 50 mg/vial (Contains ~0.3% dithiothreitol)	66 kDa
• Alcohol Dehydrogenase from yeast, Catalog Number A8656, 25 mg/vial	150 kDa
• β-Amylase from sweet potato, Catalog Number A8781, 15 mg protein/vial (Contains ~15% NaCl, 4% glucose, and 1% dithiothreitol)	200 kDa
• Blue Dextran, Catalog Number D4772, 50 mg/vial	2,000 kDa

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the kit at –20 °C.

Procedure

Use of MWGF200 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 Sephacryl® S-200-HR (Catalog Number S200HR) 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 A_{280} 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 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.
   Notes: Mixing blue dextran with kit standards or sample proteins is not recommended since many proteins bind to blue dextran.

   Prepare protein standards and blue dextran fresh.[3] 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 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 Concentration
Albumin, A8531	10 mg/ml
Alcohol dehydrogenase, A8656	5 mg/ml
β-Amylase, A8781	4 mg/ml
Carbonic anhydrase, C7025	3 mg/ml
Cytochrome c, C7150	2 mg/ml

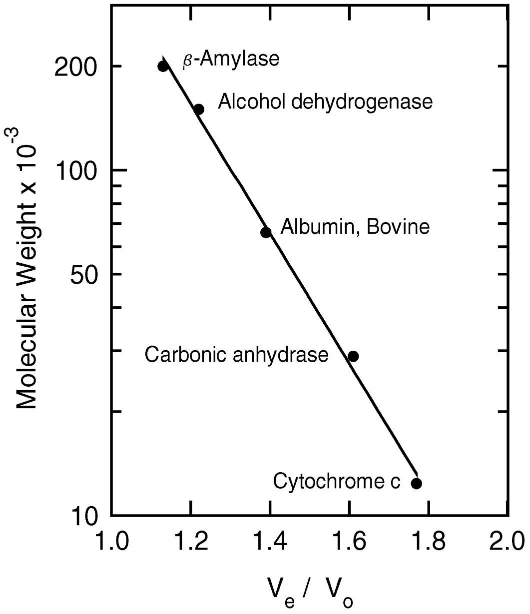
   The following proteins may be mixed and run together on the columns:

   Cytochrome c and β-amylase
   Carbonic anhydrase and alcohol dehydrogenase

   Apply 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 semilog paper (see Figure 1).

Figure 1.
Typical calibration curve obtained with proteins from the MWGF200 Kit run on Sephacryl S-200-HR.



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.

References

1. Whitaker, J.R., Anal. Chem., **35,** 1950 (1963).
2. Andrews, P., Biochem. J., **91,** 222 (1964).
3. Marshall, J.J., J. Chromatog., **53,** 379 (1970).

Sephacryl is a registered trademark of GE Healthcare.

FF,MAM,KMR 11/10-1