Gel Filtration Standard

Instruction Manual

Catalog # 1511901

BIO-RAD

Table of Contents

Section 1
Gel Filtration Standard

1.1 Introduction

Bio-Rad's Gel Filtration Standard is a lyophilized mixture of molecular weight markers ranging from 1,350 to 670,000 Da. It is a calibration standard for gel filtration/size exclusion chromatography (SEC) columns used in protein purification and analysis under nondenaturing conditions. It is appropriate for gels with exclusion limits approximately 60,000 to 5,000,000 Da. The mixture contains thyroglobulin, γ-globulin, ovalbumin, myoglobin, and vitamin B12. Both vitamin B12 and myoglobin are visible when applied to a column and provide a means of ensuring that the column is properly packed and that the sample is eluting evenly. The standard is supplied as a set of 6 vials. Each vial of the lyophilized mixture is reconstituted with 0.5 ml deionized water prior to use. The total protein content is approximately 18 mg per vial.

1.2 Instructions

Rehydrate the vial by adding 0.5 ml of deionized H_2O. Swirl gently to mix and allow the vial to stand in ice for 2–3 min. Swirl the vial again and apply the appropriate volume of standard to the column (see following recommendation). The hydrated mixture should be kept at 2–8°C, for up to two weeks. For HPLC applications, the standard should be centrifuged before application to remove any fine particulates.

Table 1. Gel Filtration Standard components.

Component	Molecular Weight*	Amount per Vial, mg
Thyroglobulin (bovine)	670,000	5.0
γ-globulin (bovine)	158,000	5.0
Ovalbumin (chicken)	44,000	5.0
Myoglobin (horse)	17,000	2.5
Vitamin B12	1,350	0.5
Total		18

* Estimates of molecular weights from:

1. Sober HA, ed. (1968). CRC Handbook of Biochemistry (Cleveland: Chemical Rubber Company).
2. Windholz M, ed. (1976). Merck Index, 9th edition (New Jersey: Merck and Company, Inc.).

1.3 Recommended Volume of Standard

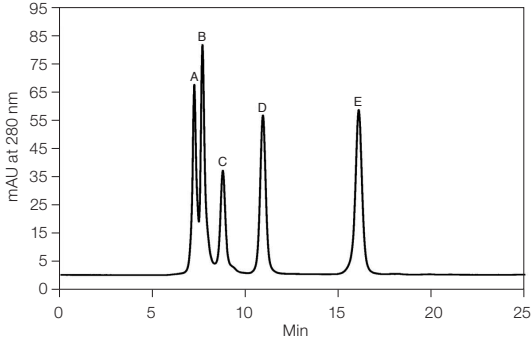
The general recommendation is to load no more than 1% of the column volume to avoid peak broadening effects. To achieve an absorbance of about 0.1–0.2 OD at 280 nm with various column sizes, use a volume of sample proportional to the total column volume. For example, a column of 170 ml total volume would require about 0.5 ml of standard, while a column of 40 ml would require about 0.125 ml. Actual chromatographic separations will depend upon chromatographic techniques and column efficiency.

Table 2. Recommended volume of standard.

Column Volume, ml	Volume of Standard, ml
100–200	0.5
50–100	0.25
25–50	0.125

Figure 1 shows the results achieved with ENrich™ SEC Columns. For HPLC size exclusion columns (10 x 300 mm), a 20 µl injection loop will give peaks with absorbance at 280 nm of about 0.75.

ENrich SEC 70 Media



ENrich SEC 650 Media

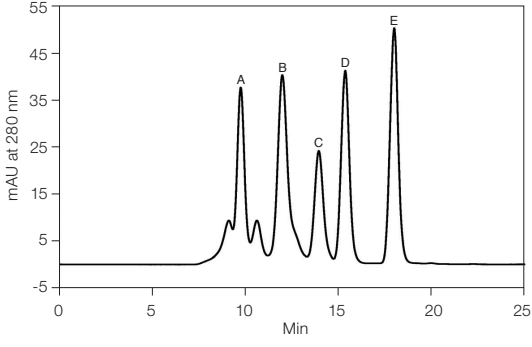


Fig. 1. Typical chromatograms for ENrich SEC Media. Detection at 280 nm. Absorbance at 280 nm may be greater depending on sample concentration. Time may change depending on flow rate. **A**, thyroglobulin; **B**, gamma globulin; **C**, ovalbumin; **D**, myoglobin; **E**, vitamin B12.

1.4 Shelf Life

The lyophilized standard is stable for at least 1 year when stored at 2–8°C. The shelf life may be extended if stored at a lower temperature.

1.5 Storage

The lyophilized standard may be stored at 2–8°C or lower. The reconstituted standard should be aliquoted and frozen at 0°C or lower and is stable for 5 years.

Section 2
Ordering Information

Catalog #	Description
1511901	**Gel Filtration Standard**, 6 vials lyophilized mixture
Columns	
7801070	**ENrich SEC 70 Column**, 10 x 300 mm, 24 ml
7801650	**ENrich SEC 650 Column**, 10 x 300 mm, 24 ml

Bio-Rad Technical Support

The Bio-Rad Technical Support department is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific time.
Phone: 1-800-424-6723, option 2
Email: Support@Bio-Rad.com (U.S./Canada only)

For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact us link at **bio-rad.com**.

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