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# Gel Filtration Standard

Instruction Manual

Catalog #  
151-1901

**BIO-RAD**

# Table of Contents

Section 1	Introduction.....	1
1.1	Instructions.....	1
1.2	Recommended Volume of Standard.....	2
1.3	Shelf Life.....	5
1.4	Storage.....	5
Section 2	Ordering Information.....	5

# Section 1

## Introduction

Bio-Rad's gel filtration standard is a lyophilized mixture of molecular weight markers ranging from 1,350 to 670,000 daltons. It is a calibration standard for gel filtration/size exclusion chromatography (SEC) columns used in protein purification and analysis under nondenaturing conditions, and is appropriate for gels with exclusion limits approximately 60,000 to 5,000,000 daltons. The mixture contains thyroglobulin,  $\gamma$ -globulin, ovalbumin, myoglobin, and vitamin B<sub>12</sub>. Both vitamin B<sub>12</sub> 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.

### 1.1 Instructions

Rehydrate the vial by adding 0.5 ml of deionized H<sub>2</sub>O. 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
$\gamma$ -globulin (bovine)	158,000	5.0
Ovalbumin (chicken)	44,000	5.0
Myoglobin (horse)	17,000	2.5
Vitamin B <sub>12</sub>	1,350	<u>0.5</u>
		18

\*Estimates of molecular weights from:

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

## 1.2 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 to 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**

<b>Column Volume</b>	<b>Volume of Standard</b>
100–200 ml	0.5 ml
50–100 ml	0.25 ml
25–50 ml	0.125 ml

Figure 1 shows the results achieved with Bio-Sil® SEC HPLC columns. For HPLC size exclusion columns (7.8 x 300 mm), a 20 µl injection loop will give peaks with absorbance at 280 nm of about 0.75.

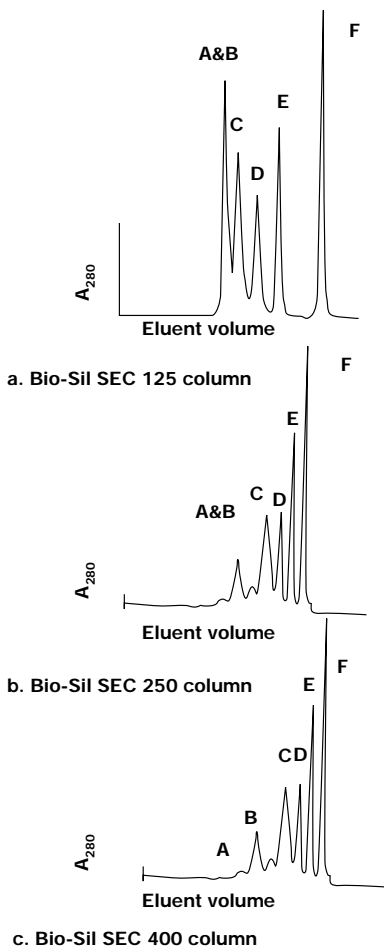


Fig. 1. Separation of standard on HPLC size exclusion columns.

**Fig. 1.**

- a. Bio-Sil SEC 125 column (exclusion limit\*  $\approx$  80,000).
- b. Bio-Sil SEC 250 column (exclusion limit  $\approx$  300,000).
- c. Bio-Sil SEC 400 column (exclusion limit  $\approx$  800,000).

**Peaks:**

- A. Protein aggregates (void peak)
- B. Thyroglobulin
- C.  $\gamma$ -globulin
- D. Ovalbumin
- E. Myoglobin
- F. Vitamin B<sub>12</sub>

**Conditions**

Buffer	0.05 M Na <sub>2</sub> HPO <sub>4</sub> , 0.05 M NaH <sub>2</sub> PO <sub>4</sub> , 0.15 M NaCl, pH 6.8
Column	300 x 7.8 mm
Flow rate	1.0 ml/min
Sample	20 $\mu$ l
Chart speed	1 cm/min
Detection	UV @ 280 nm; 1.0 AUFS

\*Nominal exclusion limits given for globular proteins.

### 1.3 Shelf Life

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

### 1.4 Storage

The lyophilized standard may be stored at 2 to 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

<b>Catalog #</b>	<b>Description</b>
151-1901	Gel Filtration Standard, 6 vials lyophilized mixture
125-0060	Bio-Sil SEC 125-5 Column, 7.8 x 300 mm
125-0065	Bio-Sil SEC 125-10 Column, 7.5 x 600 mm
155-0200	Bio-Sil SEC 125-10 Column, 21.5 x 600 mm
125-0062	Bio-Sil SEC 250-5 Column, 7.8 x 300 mm
125-0066	Bio-Sil SEC 250-10 Column, 7.5 x 600 mm
155-0201	Bio-Sil SEC 250-10 Column, 21.5 x 600 mm
125-0064	Bio-Sil SEC 400-5 Column, 7.8 x 300 mm
125-0067	Bio-Sil SEC 400-10 Column, 7.5 x 600 mm
155-0202	Bio-Sil SEC 400-10 Column, 21.5 x 600 mm

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