



Protein Standard for Hydrophobic Chromatography Instruction Manual

**Catalog Number
151-1905**

BIO-RAD

For Calibration of Hydrophobic Columns

The Protein Standard for Hydrophobic Chromatography is a lyophilized mixture of proteins which exhibit different hydrophobic behavior. It may be used as a calibration standard for HIC columns for analysis under nondenaturing conditions, and is appropriate for calibrating some reverse phase columns as well. The mixture contains cytochrome c, myoglobin and lysozyme. The standard is supplied as a set of 6 vials of lyophilized protein mixture. The total protein content is approximately 3 mg per vial.

Instructions

Rehydrate the standard by adding 0.5 ml of starting buffer to the vial. Swirl gently to mix and allow the vial to stand for 2–3 minutes. Swirl the vial again; centrifuge or filter to remove fine particulates before injection.

Protein Standard Components

Component	Molecular Weight
Cytochrome c	12,000
Myoglobin (horse)	17,000
Lysozyme	14,000

Recommended Volume of Standard

Figure 1 shows separations of the standard using Hi-Pore® RP-304 HPLC columns. A volume of 20 µl standard was applied to each column. The protein peaks give absorbance at 280 nm from about 0.03 to 0.1 absorption units.

Conditions
Column: Reversed Phase
Hi-Pore RP-304, 250 x 4.6 cm
Sample: 20 µl
Eluant: A. 0.1% TFA
B. 0.1% TFA, 95% acetonitrile
25-50% B linear gradient, 30 min
Flow Rate: 1.5 ml/min
Detection: UV Monitor @ 280 nm
0.08 AUFS

Peaks:
1. Cytochrome c
2. Myoglobin
3. Lysozyme

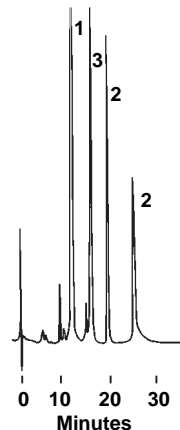


Fig. 1. Separation of standard.

Product Information

Catalog Number	Product Description
151-1905	Protein Standard for Hydrophobic Chromatography , 6 vials lyophilized mixture
125-0550	Hi-Pore RP-304 Reversed Phase HPLC Column , 250 x 4.6 mm
125-0131	Micro-Guard® Holder
125-0134	Micro-Guard Refill Cartridges for Hi-Pore Columns