INSTRUCTIONS for Poly(A)-Sepharose 4B

Poly(A)-Sepharose 4B is an adsorbent for affinity chromatography of a wide range of molecules that exhibit specific, reversible affinity for polyadenylic acid. These include:

- mRNA-binding proteins
- poly(A)-binding RNA
- viral RNA
- DNA-dependent RNA polymerases
- antibodies to nucleic acids
- tubulin assembly protein

Poly(A)-Sepharose 4B is formed by the covalent coupling of polyadenylic acid chains (approximately 100 nucleotides long) to Sepharose 4B by the cyanogen bromide method. The resulting multi-point covalent attachment via the N'-amino groups of the base residues is more stable than single-point attachment by terminal free phosphate groups. The long poly(A) chain acts as its own spacer, ensuring good binding capacity.

The concentration of coupled poly(A) is approximately 0.25 mg per mL of swollen gel. The binding capacity for 2.5 S poly(U) is not less than 0.50 mg per mL of swollen gel.

Poly(A)-Sepharose 4B is supplied as a freeze-dried powder in packs of 5 g, equivalent to approximately 20 mL of swollen gel. Additives have been included to preserve the swelling characteristics of the gel.

The freeze-dried powder should be kept dry at 4-8 °C. The swollen suspension can be stored at 4–8 °C in the presence of a bacteriostatic agent.

Methods for Using Poly(A)-Sepharose 4B

Because Poly(A)-Sepharose 4B can be used to separate a wide range of substances, it is not possible to define any single optimum procedure. However, the following methods provide a basis for further experimentation.

Swelling and Washing the Gel

1. Swell the required amount of freeze-dried powder for 15 minutes in 0.1 M NaCl, pH 7.5.
2. Pack the gel in a column.
3. Wash the gel with 0.1 M NaCl solution (100 mL per gram dry powder) and then with 90% formamide, 0.01 M phosphate, 0.01 M EDTA, pH 7.5.
4. Equilibrate the gel with five bed volumes of starting buffer.

Starting Buffer

Proteins
Use a low ionic strength buffer to facilitate binding, such as 0.01 M Tris-HCl, pH 7.4. Low molecular weight thiol compounds, EDTA, and metal ions can be used if required.

Unless RNase is added to protein samples, nucleic acids present in the sample can bind to the adsorbent. The elution patterns of RNase-treated and untreated samples might therefore be different. Use RNase treatment when working with RNA polymerases.

Nucleic acids
Use a high ionic strength buffer to minimize protein binding: 25% formamide, 0.7 M NaCl, 0.05 M Tris-HCl, 0.01 M EDTA, pH 7.5.

Lauroyl sarcosine or another suitable agent can be used to inhibit ribonucleases that might hydrolyze the nucleic acids or the poly(A) chain.

Elution

Proteins
Increased ionic strength, formamide treatment, reduced pH, or treatment with SDS or 7M guanidine hydrochloride

Nucleic acids
Conditions that destabilize hydrogen bonds, such as formamide treatment, increased temperature, or increased ionic strength

Regeneration

1. Wash with several bed volumes of elution buffer or with 10 mM NaOH (pH 12) for 10 minutes. Avoid prolonged exposure to alkali.
2. Requilibrate the gel with starting buffer.

Ordering Information

Product	Pack size	Product Code
Poly(A)-Sepharose 4B	5 g	17-0860-01