INSTRUCTIONS for Poly(U)-Sepharose 4B

Poly(U)-Sepharose 4B is a reliable adsorbent for specific and reversible binding of:

- messenger RNAs (mRNA)
- reverse transcriptases
- interferons
- nucleic acids from plants

The interaction with mRNA results from biospecific hybridization between a synthetic immobilized chain of polyuridylic acid, poly(U), and the complementary poly(A) sequence that is a characteristic feature of almost all mRNA molecules. High recoveries of pure mRNA are obtained even when the mRNA contains short poly(A) strands. The matrix, Sepharose 4B, exhibits negligible nonspecific adsorption effects and provides good flow properties for rapid separation of pure mRNA.

Poly(U)-Sepharose 4B is formed by the stable covalent coupling of long poly(U) chains (approximately 100 nucleotides long) to Sepharose 4B by the cyanogen bromide method. This method gives multiple-point covalent attachment via the tautomeric enolate form of the nucleotides and not by the less favourable single-point esterification through the terminal free phosphate group. This ensures a firmly bonded stable affinity support.

The concentration of coupled poly(U) is approximately 0.5 mg per mL swollen gel. The binding capacity for mRNA from KB cell polysomal preparations is approximately 150 µg mRNA per mL swollen gel (equivalent to approximately 5 absorbance units). Recoveries of mRNA are approximately 90%. It is possible to process 60–90 absorbance units of ribosomal material per mL gel.

Poly(U)-Sepharose 4B is supplied as a freeze-dried powder in packs of 5 g, equivalent to approximately 20 mL 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. Storage for 1 year results in loss of approximately 2% nucleotide material. However this is due to cleavage of monomers from the polynucleotide rather than to loss of entire chains and binding capacity for mRNA is unaffected. Storage of Poly(U)-Sepharose 4B in suspension (pH 7.7, 5 °C) for 6 months results in no more than 2% loss of nucleotide material.

Swelling and Washing the Gel

1. Swell the required amount of dry gel in 0.1 M NaCl, pH 7.5.
2. Wash the gel on a sintered glass filter using 100 mL of 0.1 M NaCl, pH 7.5, per gram of dry gel.
3. Pack the gel in a suitable column.
4. Wash the gel with the same volume of eluting buffer.
5. Equilibrate with an equivalent volume of the starting buffer: 25% formamide, 0.7 M NaCl, 50 mM Tris-HCl, 10 mM EDTA, pH 7.5.

Sample Preparation

1. Prepare polysomal samples in detergent solution (1% lauroyl-sarcosine, 30 mM EDTA). The detergent inhibits ribonucleases that might hydrolyze the poly(U) chain. Other ribonuclease inhibitors can be used.
2. Dilute the sample five-fold with starting buffer and apply it to the column.
3. Wash the column with ten bed volumes of starting buffer.

Elution

The biospecific interaction between Poly(U)-Sepharose 4B and mRNA involves hydrogen bond formation. Any conditions that break hydrogen bonds should cause elution. Formamide efficiently dissociates the poly(U):poly(A) complex.
For the elution buffer, use 90% formamide, 10 mM potassium phosphate, 10 mM EDTA, 0.2% lauroyl-sarcosine, pH 7.5. Alternatively, you can use an increase in temperature.

Monitoring

Monitor the chromatography by radioactive labeling of the preparations or by UV monitoring at 260 nm. However, the 90% formamide gives a rather high background reading, making it necessary to use an instrument with reference cells, such as the GE Healthcare Biosciences UV-1 monitor.

Regeneration

Wash with the elution buffer for several bed volumes. You can also wash for 10 minutes with 10 mM NaOH (pH 12). Prolonged exposure to alkali should be avoided. After washing, equilibrate the gel with starting buffer.

Ordering Information

Product	Pack size	Product Code
Poly(U)-Sepharose 4B	5 g	17-0610-01