Product Information

Heparin Agarose

Type I

H6508

Storage Temperature 2-8 °C
Do Not Freeze

Product Description

This resin is a cross-linked 4% beaded agarose. The method of activation is through cyanogen bromide attachment, which results in an amino spacer of one atom.

The agarose suspension is in 0.5 M NaCl containing preservative. The molecular weight range of the heparin is between 25-35 kDa (based on low angle laser light scattering) and 18-23 kDa (based on size exclusion chromatography).

The amount of heparin attached to the agarose is determined in the following manner. Prepare a heparin standard in 0.2% NaCl. Prepare a standard curve from 0.1 to 10 µmoles heparin. Add 2.5 mL of 0.005% toluidine blue in 0.01 M HCl and 0.2% NaCl. Add additional 0.2% NaCl solution to bring the volume to 5 mL. For the resin samples, take 0.2 to 0.8 mL of resin. Add 2.5 mL of toluidine blue solution and sufficient 0.2% NaCl to make a final volume of 5 mL. Mix samples and standards for 10-15 minutes. Add 5 mL of hexane and mix. Transfer the aqueous layer to a cuvette and read absorbance in a spectrophotometer at 631 nm. From the standard curve calculate µmole of heparin per mL of packed gel.[1]

Heparin-agarose can be used for separation of various RNA polymerases and protein kinases. A typical buffer used for such applications would be 50 mM Tris-HCl, pH 7.9, 6 mM $MgCl_2$, 0.1 mM PMSF, 10 mM 2-mercaptoethanol, 0.05% (v/v) monothioglycerol, and 20% (v/v) glycerol with varying amounts of ammonium sulfate for the elution gradient.[2.3.4]

This resin is recommended for all-purpose enzyme purification (see the Procedure section for general guidelines). Applications found in the research literature include: Purification of reverse transcriptase;[5] purification of phospholipase A1;[6] purification of viruses.[7]

Heparin-agarose Type I has been used in the purification of recombinant adeno-associated virus. When combined with preliminary purification steps (use of nonionic iodixanol gradients), 50% recovery of rAAV from a crude lysate can be achieved. The resulting vector is more than 99% pure.[7] Heparin-agarose Type II was found to be less selective in binding many cellular proteins along with the virus.

Precautions and Disclaimer

For R&D use only. Not for drug, household or other uses.

Preparation Instructions

This agarose suspension can be washed with water followed by the appropriate buffer to remove the preservative before use.

Storage/Stability

This is a very stable agarose. If the resin is stored in a 2-8 °C refrigerator in a buffer solution containing preservative, it should be useful and stable for up to 5 years. **Do not freeze.** Freezing will damage the agarose resin.

Procedure

Suggestions for general use: 2.5 mL bed volume.

Equilibration buffer: 0.01 M Tris-HCl, pH 7.5 to 8.0 (Other buffer systems may be substituted if the target protein is unstable in Tris buffers). Buffer additions are acceptable and at times essential for protein stability (Mercaptoethanol, EDTA, for example).

Elution buffer: 0.01 M Tris-HCl, pH 7.5-8.0, with 1.5 M NaCl. Alternative salts may be used [KCl, $CaCl_2$, NH_4Cl, $(NH_4)_2SO_4$]. Specific eluants can also be used: (5-50 mM) nucleotides; cofactors; coenzymes; chaotropic agents such as (0.5 M to 6 M) urea, guanidine, sodium thiocyanate; TRITON® X-100 (0.1-2%); ethylene glycol (0.1-2%); or pH shifts (use with care: from 3.2 to 10).

Sample Preparation

Centrifugation: Eliminate particles and minimize lipid or lipoprotein content (this will aid in resin cleaning and extend column life).

Concentration: Between 1-10 mg/mL. Equilibration to column conditions by dialysis, desalting columns, diafiltration, or dilution.

Procedure for Use

Recommended running temperature 3-8 °C

1. Equilibrate column with 5-10 column volumes of appropriate buffer for the target protein.
2. Load the protein solution onto the column.
3. Wash the load into the column with a small volume (0.1-0.5 mL) of equilibration buffer.
4. Continue washing to remove unbound protein. Washing may require 3-10 column volumes for complete removal of free protein.
5. Elute bound protein with the chosen elution buffer.

   Note: Some proteins may require severe conditions to elute from columns.
6. Assay eluted fractions for target protein.
7. Clean the column.

Cleaning of the Column

Wash the column with 10 column volumes of each:

1. 0.1 M borate, pH 9.8 with 1 M NaCl
2. 0.1 M borate, pH 9.8
3. Deionized or distilled water
4. 2.0 M NaCl

For removal of strongly bound impurities, the column can be washed with:

1. 2 volumes of 2 M ammonium sulfate
2. 3 volumes of 1 M Tris, pH 8.0, containing 6.0 M urea (or alternatively containing 6 M guanidine hydrochloride)
3. 3 volumes of distilled water[4]

Variables that will affect protein binding capacities

- Lack of consistency in protein solutions, changes in specific activity, or different contaminating proteins within your sample solution that may inhibit or compete for binding sites.
- Inconsistencies in equilibration of resins or buffer preparation (ionic strengths incompatible to protein binding).
- Inconsistencies in elution techniques.
- Condition of the resin (such as age or effectiveness of cleaning).

References

1. Smith, P. K., et al., Colorimetric Method for the Assay of Heparin Content in Immobilized Heparin Preparations. Anal. Biochem., 109, 466-473 (1980).
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3. Chaiken, I. M., et al., International Symposium on Affinity Chromatography and Biological Recognition, 5th ed., eds., New York (2012).
4. Dean, P.D.G., et al., Affinity Chromatography, A Practical Approach, 125-133 (1985).
5. J. Virol. Methods, 1, 157 (1980).
6. Waite, M., et al., Phospholipases A1 from Lysosomes and Plasma Membranes of Rat Liver. Meth. Enzym., 71, 687 (1981).
7. Zolotukhin, S., Gene Therapy, 6, 973-985 (1999).

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