Affi Gel® Heparin Gel for Protein Purification

Affi-Gel heparin gel is a ready-to-use affinity support for the purification of a wide range of proteins. It consists of heparin coupled via amide bonds to crosslinked agarose beads. As a result of this coupling, the gel is stable in all commonly used buffers and denaturants. No loss of activity is apparent after multiple cycles of use and regeneration. Affi-Gel heparin gel contains 1.0 (±0.2) mg heparin/ml gel, and will bind at least 1.2 mg human antithrombin III/ml gel. It is supplied hydrated in PBS, with 0.02% sodium azide as a preservative.

Heparin is a linear glycosaminoglycan, composed of mostly sulfated 1-4 linked glucosamine and glucuronic acid residues. It binds a variety of enzymes and other proteins, either ionically or by a more specific enzyme-inhibitor (or enzyme-activator) interaction. Heparin binding has been demonstrated in such protein classes as coagulation factors and other plasma proteins, polynucleotide polymerase, nuclease, endonuclease, lipase, lipoproteins, and proteases. These, and other similar proteins, may lend themselves to affinity purification on Affi-Gel heparin gel.

For an extensive review of heparin-agarose affinity chromatography, see Reference 1. Reference 2 reviews heparin-protein interactions.

Instructions for Use

1. Pour column of Affi-Gel heparin gel. (The capacity of the gel for various proteins is typically 0.2-2.0 mg/ml gel.)
2. Equilibrate the column with 3-5 bed volumes of the application buffer (a common buffer is PBS, 0.01 M KH_2PO_4, 0.15 M NaCl, pH 7.4).
3. Apply the sample in application buffer at a linear flow rate of 10-20 cm/hr.
4. Wash the column with application buffer until the O.D. $_{280}$ nm of the eluant is the same as the application buffer.
5. Elute the bound protein with application buffer plus 1.5 M NaCl.

   When more than one protein binds to the column, resolution may be achieved by a linear gradient of 0.15-2.0 M NaCl in the application buffer, or by stepwise elution with increasing concentrations of salt in the application buffer. Elution with heparin (at a concentration of 1–10 mg/ml in the application buffer) may selectively elute some proteins.
6. Regenerate the column with 2-3 bed volumes of 8 M urea, 1.5 M NaCl in PBS. Follow the urea wash with 3-5 bed volumes of application buffer. The column is now ready for re-use.
7. When not in use, store the column at 4 °C with 0.02% sodium azide added as a preservative.

Application

Purification of Human Thrombospondin[3]

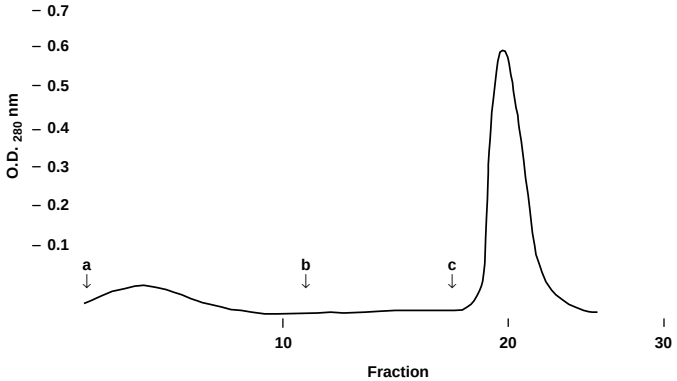

Fig. 1. Thrombospondin was purified by a modification of the method of Lawler, et al.[4] After fibrin removal, crude thrombospondin from 8 units of fresh human platelet concentrate was applied to a Bio-Gel® P-300 column (6 x 45 cm) in 0.02 M Tris, 0.25 M NaCl, 2.5 mM EDTA, pH 7.4. Void volume fractions were pooled and applied (a) to the Affi-Gel heparin column (1 x 5 cm). The Affi-Gel heparin column was washed with the same buffer until the $O.D._{280}$ returned to baseline (b). NaCl concentration was established, initially, by eluting with a NaCl gradient (0.15 to 2.0 M NaCl in Tris, EDTA buffer). Data courtesy of M.J. Doyle and D.F. Mosher, Department of Medicine, University of Wisconsin.

References

1. Farooqui, A. A., *J. Chromatography,* **184,** 335 (1980).
2. Jaques, L. B., *Pharmacological Reviews,* **31,** 99 (1980).
3. Doyle, M. J. and Mosher, D. F., Dept. of Medicine, University of Wisconsin. We thank M. Doyle and D. Mosher for their assistance in the evaluation of this product.
4. Lawler, J. W., Slayter, H. S. and Coligan, J. E., *J. Biol. Chem.,* **253,** 8609 (1978).

Ordering Information

Catalog Number	Product Description
153-6173	**Affi-Gel Heparin Gel,** 40 ml

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