Product Information

# Heparin-Agarose

1:1 suspension in a 20% ethanol solution

**H0402**

## Product Description

Heparin is a polysaccharide composed of equimolar quantities of glucosamine and glucuronic acid, alternatively linked by α(1→4) glycosidic bonds.[1] A certain number of its hydroxyl groups are esterified with sulfuric acid moieties. Overall, the molecule possesses a single reducing sugar terminus. Under basic pH conditions, this terminal sugar will be in the reduced or aldehyde form.

This product is prepared through the attachment of an oxygen on the heparin molecule to an amine on the spacer arm of epichlorohydrin-activated, 4% cross-linked beaded agarose.[2] This aldehyde is reacted with the amine of the resin spacer arm to form a Schiff base, which is then efficiently reduced by the pyridine borane (reductive amination) to prepare the heparin-agarose linkage.

As a result of its composition and its biochemical role, heparin can bind a number of proteins, enzymes, and polycationic organic compounds. Heparin can also bind alkaloids, antibiotics, stains, and hormones. These interactions may be specific, as with certain coagulation factors, or may be due to more complex ionic interactions. Several major groups of proteins can be purified on heparin-agarose:

1. Coagulation factors such as ATIII,[3,4] Factor IX, Factor VII, Factor XI, Factor XII, and Xlla.
2. Lipoprotein lipases,[5] which form ionic complexes with heparin. There are numerous reports on the purification of lipoprotein lipases from serum, mammalian heart, adipose tissue, and bovine milk.
3. Lipoproteins (LDL, VLDL, VLDL apoprotein, and HDL), which may form an insoluble complex with heparin in the presence of divalent cations.[6-8] This property is exploited in the separation of serum lipoproteins on immobilized heparin (lipoprotein elimination from serum to reduce interference with enzymatic assays).
4. Growth hormones and growth factors.[9-12]
5. DNA- and RNA-related enzymes, since heparin is an inhibitor of DNA and RNA polymerases, and interacts with numerous DNA- and RNA-dependent enzymes. These properties are used to purify a wide variety of enzymes (polymerases and restriction endonucleases, for example).[13]

Immobilized heparin has been also used for the purification of various other biomolecules, such as:

- Fibronectin and fibronectin fragments[14,15]
- Hormone receptors[16-17]

Several theses and dissertations have cited use of this specific heparin-agarose H0402 product in their research protocols.[18-19]

## Reagent

This product is supplied as a 1:1 suspension of beads to solvent, where the solvent is 20% ethanol.

The heparin-agarose has a binding capacity of 4-8 mg of antithrombin III (ATIII) per mL of resin.

## Procedure

### Procedure for determination of ATIII

1. Approximately 0.8 mL of resin is packed into a 3 mL syringe column.
2. Wash the resin with 10 volumes of running buffer (0.01 M Tris-HCl, at pH 7.5, with 0.15 M NaCl).
3. The antithrombin III solution is loaded onto the column, and 10 mL fractions are collected.
4. After loading, the column is washed with at least 5 column volumes of running buffer, until the $A_{280}$ has dropped below 0.01.
5. The ATIII is eluted with 4-5 column volumes of elution buffer (0.01 M Tris-HCl, pH 7.5, with 2 M NaCl), and ½-column volume fractions are collected. The $E^{0.1\%}$ at 280 nm for ATIII is 0.65.

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## References


1. *The Merck Index*, 12th Ed., Entry# 4685.
2. Pirotta, V., and Bickle, T.A., *Meth. Enzymol.*, **65**, 89-95 (1980).
3. Sakaki, H. *et al.*, *J. Chromatogr. A*, **400**, 123-132 (1987).
4. Mitra, G., *et al.*, *Biotechnol. Bioeng.*, **28(2)**, 217-222 (1986).
5. Sivaram, P. *et al.*, *J. Biol. Chem.*, **267(23)**, 16517-16522 (1992).
6. Iverius, P.H., *J. Biol. Chem.*, **247(8)**, 2607-2613 (1972).
7. Pan, Y.T., *et al.*, *Arch. Biochem. Biophys.*, **189(2)**, 231-240 (1978).
8. Quarfordt, S.H., *Biochem. Biophys. Res. Commun.*, **83(3)**, 786-793 (1978).
9. Lefevre, C. *et al.*, *EMBO J.*, **6(4)**, 971-981 (1987).
10. Zarnegar, R., and Michalopoulos, G., *Cancer Res.*, **49(12)**, 3314-3320 (1989).
11. Brigstock, D.R. *et al.*, *Biochem. J.*, **266(1)**, 273-282 (1990).
12. Berry, S.A. *et al.*, *Mol. Endocrinol.*, **8(12)**, 1714-1719 (1994).
13. Ber, E., Muszyńska, G., Tarantowicz-Marek, E., and Dobrowolska, G., in *Affinity Chromatography and Biological Recognition* (Chaiken, I.M., Wilchek, M., and Parikh, I., eds.), Part VI (Applications in Separation and Biotechnology). Academic Press (New York, NY), pp. 455-459 (1983).
14. Millis, A.J.T. *et al.*, *Proc. Nat. Acad. Sci. USA*, **82(9)**, 2746-2750 (1985).
15. Brew, S.A., and Ingram, K.C., *J. Tissue Culture Methods*, **16**, 197-199 (1994).
16. Molinari, A.M. *et al.*, *Proc. Nat. Acad. Sci. USA*, **74(11)**, 4886-4890 (1977).
17. Jiang, M.S., and Hart, G.W., *J. Biol. Chem.*, **272(4)**, 2421-2428 (1997).
18. Germon, Anna L., "Identification of Protein Disulphide-Isomerase A3 Dependent Proteins from the Secretome of MDA-MB-231 Breast Cancer Cells". University of Bristol, M.Sc. thesis, p. 31 (2019).
19. Neely, Eric, "The Development of Local-acting Biologics to Treat Rheumatoid Arthritis". University of Toronto, Ph.D. dissertation, p. 74 (2019).


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