

## Product Information

Product Number **54836**

### Product Description

This product is supplied as 5×, 1 ml column and has a binding capacity of approximately 3 mg of antithrombin III (ATIII) per ml of resin.

This product is prepared through the coupling of an oxygen on the heparin molecule to an amine on the spacer arm of epichlorohydrin-activated, 4% cross-linked beaded agarose.<sup>1</sup>

Heparin is a polysaccharide composed of equimolar quantities of glucosamine and glucuronic acid, alternatively linked by  $\alpha(1\rightarrow4)$  glycosidic bonds.<sup>2</sup> A certain number of its hydroxyl groups are esterified with sulfuric acid moieties and 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 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 has the ability to bind a number of proteins, enzymes, and polycationic organic compounds. It 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. Seven major groups of proteins can be purified on heparin-agarose:

1. Coagulation factors such as ATIII,<sup>3,4</sup> Factor IX, Factor VII, Factor XI, Factor XII, and XIIa.
2. Lipoprotein lipases, 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.<sup>5-7</sup> 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.
5. Growth factors such as FGF and ECGF.
6. 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).<sup>8</sup>
7. Other applications: immobilized heparin has been used for the purification of various other enzymes (collagenase,  $\alpha$ -L-iduronidase, hyaluronidase, and lysozyme), fibronectin, fibronectin fragments, and hormones receptors.

### Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

### 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 1/2 column volume fractions are collected. The  $E^{0.1\%}_{280}$  at 280 nm for ATIII is 0.65.

### References

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6. Pan, Y. T., et al., Binding of [3H]heparin to human plasma low density lipoprotein. Arch. Biochem. Biophys., **189(2)**, 231-240 (1978).
7. Quarfordt, S. H., The heterogeneity of rat high density lipoproteins. Biochem. Biophys. Res. Commun., **83(3)**, 786-793 (1978).
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