

# Immobilized Heparin

## Product Description

High-binding, NaOH-cleanable, stable resins to improve your affinity separations

Catalog number: [35692A](#), 10ml

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Name: **Heparin-Affarose**

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**SuperFlow**

**Superflow™ Plus**

**Ultraflow™6**

- High binding capacity
- Undetectable leaching of heparin (<0.1 ppm)
- High flow rate (up to 6,000 cm/h) on Ultraflow support
- Sanitize in 0.5 M NaOH
- [Drug Master File](#)

## Directions for Use

### The Ligand

Heparin is a naturally occurring, linear glycosaminoglycan consisting of a repeating dimer of alpha-L-dipyranoic acid 2-sulfate and 2-deoxy-2-sulphamino-a-D-glucopyranose 6-sulfate. Heparin exists in a wide range of molecular weights from 5,000-30,000 Daltons. The primary linkages are 1-4 with very little 1-6 branching. Minute amounts of other sugars are also present. Heparin is highly charged and strongly acidic.

### Binding and Elution

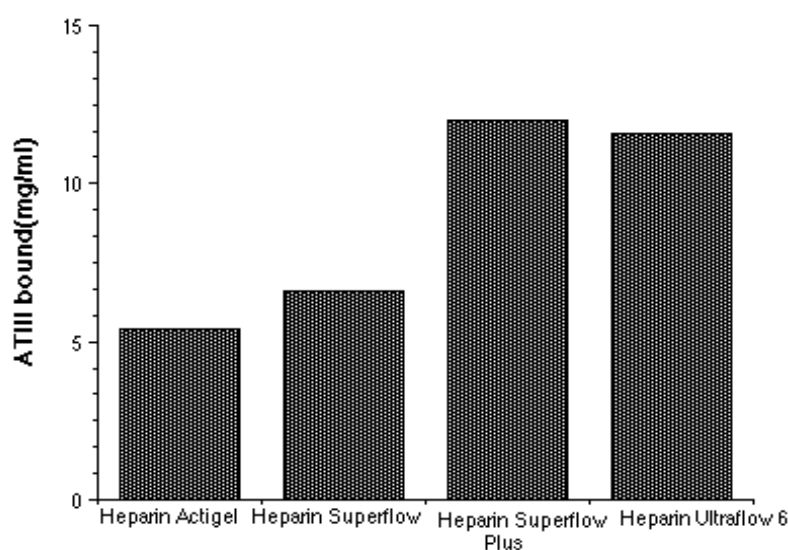
Immobilized heparin interacts with proteins by two different mechanisms: It can function as an affinity ligand, in which case the protein (such as a coagulation factor) is eluted with a buffer containing either salt or heparin. Heparin's anionic sulphate groups also give it the ability to function as a high-capacity cation exchanger. In this case, the protein is recovered by gradient elution with salt.

With heparin's unique combination of affinity for many proteins and ion exchange, good purification factors can be achieved despite relatively small differences between proteins. The nature of our immobilized heparin media make them versatile tools for the separation of many proteins.

### High Binding Capacity

Our immobilized heparin media exhibits high binding affinity for a variety of proteins. The binding capacity for human antithrombin III is in the range of 5-12 mg per ml resin.

**ATIII Binding Capacity of Heparin Media**



## No Detectable Leaching of Heparin

The ALD linkage chemistry creates exclusively uniform, stable secondary amine linkages between the reactive monoaldehyde groups and primary amino groups of the heparin. The stability of the secondary amine is so high that leaching of the heparin is not detectable at **0.1 ppm**, the sensitivity limit of the assay method.

The stability of Heparin Affarose was tested in a 6-month storage experiment in 0.1 M NaOH. The resin supernatant was sampled and tested for degradation products. Sensitivity was in the nanomole range. Over 6 months, <5% loss of binding capacity of the resin was observed.

## High Flow Rates

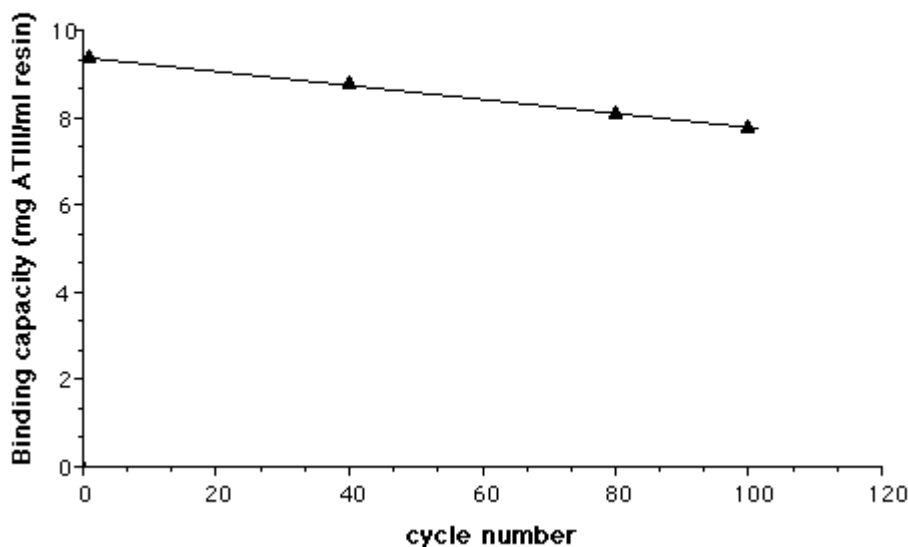
The efficiency and throughput of affinity separations can be greatly improved by combining high binding capacity with fast flow. Flow rates up to 3,000 cm/h can be achieved with Heparin Superflow. This translates into faster processing times, or smaller columns for processing the same volume of product, making Superflow particularly useful for many process-scale applications.

## Sanitization with NaOH

The utility of traditional heparin resins is limited because they cannot be sanitized with high concentrations of sodium hydroxide, the standard sanitization method for ion exchange and gel filtration media.

In contrast, our immobilized heparin media can be depyrogenated by the standard treatment using 0.5 M NaOH. Some 3-5 bed volumes are required. The resins can also be stored in 0.05 M NaOH for at least 3 months. The stability of the coupled heparin and the stable secondary amine linkage allow the use of our immobilized heparin media in the pH range 4-13.

**ATIII Binding of Heparin Superflow Plus  
on 100 Regeneration Cycles with  
0.5 M NaOH (3 hrs each)**



## Highly Reproducible Resins

Irreproducible coupling is most often due to inactivation of the activated groups, a frequent problem with traditional coupling chemistries. Using the ALD chemistry for heparin immobilization, coupling efficiency is high and very reproducible as no hydrolysis or inactivation of active groups take place during coupling in a side reaction. This provides highly reproducible resins, well-suited for both research and manufacturing. Analysis of different lots shows that variation of coupling of ligands to ALD activated media is less than 5%.

FT-35692

## Applications

Complement and coagulation factors

RNA and DNA polymerases

Restriction endonucleases

Ligases

Protein synthesis factors

Lipases and lipoproteins

Viral proteins

## Storage

The resins (as supplied) are stable for at least 2 years at 4°C. They should be stored between 2°C and 8°C at pH 7.0 with a preservative such as 0.02% merthiolate, 0.02% sodium azide or 20% ethanol. When packed into a column, the resins can also be stored in 0.05 M NaOH for extended periods of time (over 6 months).

## Specifications

	Affarose	Affarose Superflow
Agarose:	4%	6%
h-ATIII binding capacity: (mg/ml) resin	5-6	6-7
Molecular exclusion:	20 million daltons	6 million daltons
Flow rate up to:	250 cm/hr	2,200 cm/hr
Bead size:	60-160 µm	
Spacer arm:	5 atoms, hydrophilic	
Cleaning:	0.5 M NaOH	
Storage:	0.05 M NaOH	
Autoclavable:	yes	

	Affarose-Ultraflow 6	Affarose-Superflow Plus
Agarose:	6%	
h-ATIII binding capacity: (mg/ml) resin	10-12	
Molecular exclusion:	4 million daltons	6 million daltons
Flow rate up to:	6,000 cm/hr	2,200 cm/hr
Bead size:	40-160 µm	60-160 µm
Spacer arm:	5 atoms, hydrophilic	

FT-35692

Cleaning:	0.5 M NaOH
Storage:	0.05 M NaOH
Autoclavable:	yes

## Ordering Information

Product	Catalog No.	Package size
Heparin Affarose	<a href="#">35692A</a> ,	50 ml
	<a href="#">Inquire</a>	1 L
Heparin Affarose Superflow	<a href="#">Inquire</a>	50 ml
	<a href="#">Inquire</a>	1 L
Heparin Affarose Superflow Plus	<a href="#">Inquire</a>	50 ml
	<a href="#">Inquire</a>	1 L
Heparin Affarose Ultraflow 6	<a href="#">Inquire</a>	50 ml
	<a href="#">Inquire</a>	1 L

All immobilized heparin media are available in bulk pack sizes for industrial applications. Please inquire for pricing information.

## Technical and Scientific Information

### Literature

Laurent, T.C. et al. The molecular-weight dependence of the anti-coagulant activity of heparin. *Biochem. J.* 175 (1978) 691-701.

Bajaj, S.P. et al. A simplified procedure for purification of human prothrombin, Factor IX and Factor X. *Prep. Biochem.* 11 (1981) 397-412.

Fujikawa, K. et al. Characterization of bovine Factor XIIa (activated Hageman factor). *Biochemistry* 16 (1977) 4182-4188.

McKay E.J., Laurell, C.-B. The interaction of heparin with plasma proteins. *J. Lab. Clin. Med.* 95 (1980) 69-80.

Boberg, J. et al. Quantitative determination of hepatic and lipoprotein lipase activities from human post heparin plasma. *J. Lipid Res.* 18 (1977) 544-547.

Van der Mast, C. et al. Initiation of protein synthesis in eukaryotes. *Eur. J. Biochem.* 75 (1977) 455-464.

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