

Heparinase III

Heparinase III cleaves heparan sulfate exclusively, and does not cleave unfractionated heparin or low molecular weight heparins.

Non-clinical applications:

- Research reagent (glycobiology, preparation of oligosaccharide libraries, preparation of disaccharides from heparan sulfate).

Potential clinical applications:

Heparinase III selectively cleaves heparan sulphate proteoglycans from cellular surfaces and from extracellular matrices, reducing the binding of pro-inflammatory agents such as P-selectin, L-selectin, and Interleukin 8. This action of Heparinase III reduces leukocyte rolling, adhesion and extravasation.

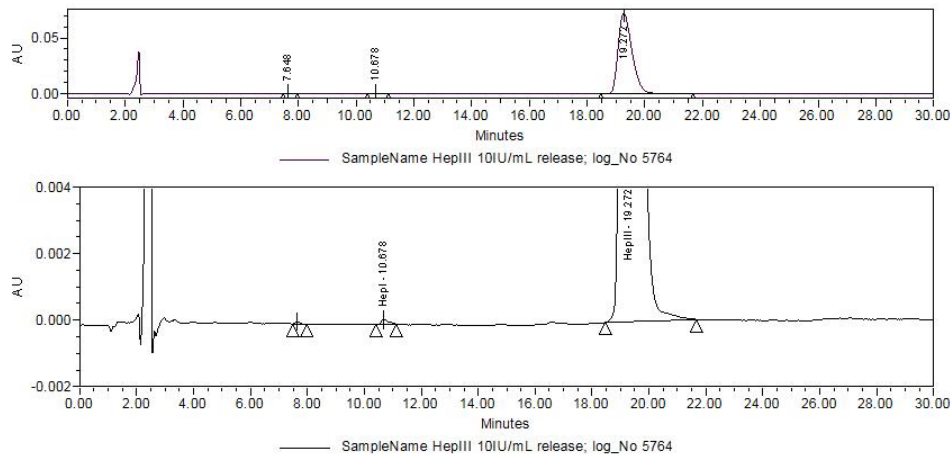
Heparinase III has been developed under the tradename Extravase™ by IBEX Pharmaceuticals for a number of potential medical applications such as:

- Protection against reperfusion injury following ischemia.
- Reduction of restenosis following angioplasty.
- Acceleration of wound healing in patients with venous ulcers.

Therapeutic application was sold to BioMarin Pharmaceuticals, Novato, California.

Heparinase III Data Sheet

Synonyms	Heparin sulfate eliminase; heparitinase I
Source	<i>Flavobacterium heparinum</i> (recombinant)
EC Number	4.2.2.8
CAS Number	37290-86-1
Catalyzed Reaction	The enzyme cleaves selectively, via an elimination mechanism, sulfated polysaccharide chains containing 1-4 linkages between hexosamines and glucuronic acid residues. The reaction yields oligosaccharide products (mainly disaccharides) containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm. The enzyme is active only towards heparan sulfate and does not cleave heparin or low molecular weight heparins.
Substrate Specificity	Heparan sulfate.
Properties	<ul style="list-style-type: none">• Molecular weight: 73,202 Da• Isoelectric point: 9.6 – 9.9• pH optimum for activity: 7 – 8• pH range for activity: 5.5 – 9• Optimal temperature range: 20 °C – 37 °C
Purity	≥95 % by reversed phase HPLC analysis.



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Specific Activity

>45 IU/mg.

One international unit (IU) is defined as the amount of enzyme that will liberate 1.0 μ mole unsaturated oligosaccharides from heparan sulfate per minute at 30 °C and pH 7.5.

Stability

- PN 50-012 (0.5 IU/vial): Expiration is 18 months from manufacturing date frozen at -70 °C in aqueous buffers containing Sodium Phosphate and Sucrose 5%.
- PN 50-120 (0.1 IU/vial): Expiration is 12 months from manufacturing date frozen at -70 °C in aqueous buffers containing Sodium Phosphate and Sucrose 5%.

Applications

- As a research reagent (glycosaminoglycan degradation).
- For the preparation of disaccharides of heparan sulfate and the preparation of oligosaccharide libraries.

Availability

A proprietary expression system for *F. heparinum* and the fermentation and isolation processes developed by IBEX Pharmaceuticals allow the production of large quantities of high purity product.

References

- Review: "Enzymatic Degradation of Glycosaminoglycans". S. Ernst et al. in *Critical Reviews in Biochemistry and Molecular Biology* (1995), 30(5): 387-444.
- "Purification and Characterization of Heparin Lyases from *Flavobacterium heparinum*". D.L. Lohse and R.J. Linhardt in *J. Biol. Chem.* (1992) 267: 24347-24355.
- "Purification and Characterization of Heparinase from *Flavobacterium heparinum*". V.C. Yang, R.J. Linhardt, H. Bernstein, C.L. Cooney and R. Langer in *J. Biol. Chem.* (1985) 260(3): 1849-1857.
- "Substrate Specificity of the Heparin Lyases from *Flavobacterium heparinum*". U.R. Desai, H. Wang and R.J. Linhardt in *Archives of Biochemistry and Biophysics* (1993) 306(2): 461-468.
- "Isolation and Expression in *Escherichia coli* of *hepB* and *hepC*, Genes Coding for the Glycosaminoglycan-Degrading Enzymes Heparinase II and Heparinase III, Respectively, from *Flavobacterium heparinum*". HongSheng Su, Françoise Blain, Roy A. Musil, Joseph J.F. Zimmermann, KangFu Gu and D. Clark Bennett, in *Applied and Environmental Microbiology* (1996): 2723-2734.
- "Heparinase III from *Flavobacterium heparinum*: Cloning and Recombinant Expression in *Escherichia coli*". R. Godavarti, M. Davis, G. Venkataraman, C. Cooney, R. Langer and R. Sasisekharan, in *Biochemical and Biophysical Research Communications* (1996) 225: 751-758.
- "Involvement of heparan sulfate and related molecules in sequestration and growth promoting activity of fibroblast growth factor". I. Vlodavsky, H-Q. Miao, P. Danagher and D. Ron, in *Cancer and Metastasis Reviews* (1996) 15(2): 177-186.

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- “Control of Cell Proliferation by Heparan Sulfate and Heparin-Binding Growth Factors”. *I. Vlodaysky, H-Q. Maio, R. Atzmon, E. Levi, J. Zimmermann, R. Bar-Shavit, T. Peretz and S. Ben-Sasson, in Thrombosis and Haemostasis (1995) 74(1): 534-540.*
- “Heparinase III Exerts Endothelial and Cardioprotective Effects in Feline Myocardial Ischemia-Reperfusion Injury”. *R. Hayward, T.O. Nossuli and A.M. Lefer, in J. Pharmacology and Experimental Therapeutics (1997) 283: 1032-1038.*
- “IBT 9302 (Heparinase III): a novel enzyme for the management of reperfusion injury-related vascular damage, restenosis and wound healing”. *P. Silver, in Exp. Opin. Invest. Drugs (1998) 7(6): 1003-1014*
- “Cellular mechanisms of heparinase III protection in rat traumatic shock”. *R. Hayward, R. Scalia, B. Hopper, J. Appel III and A. Lefer, in Am. J. Physiol. (1998) 275 (Heart Circ. Physiol. 44): H23-H30.*
- “Heparinase III limits rat arterial smooth muscle cell proliferation in vitro and in vivo”. *P. Silver, J-P. Moreau, E. Denholm, Y.Q. Lin, L. Nguyen, C. Bennett, A. Recktenwald, D. DeBlois, S. Baker, S. Ranger in Euro. J. Pharmacol. (1998) 351:79-83.*