

Heparinase I

Heparinase I cleaves heparin and to a lesser extent heparan sulfate (ratio approx. 3:1).

Non-clinical applications:

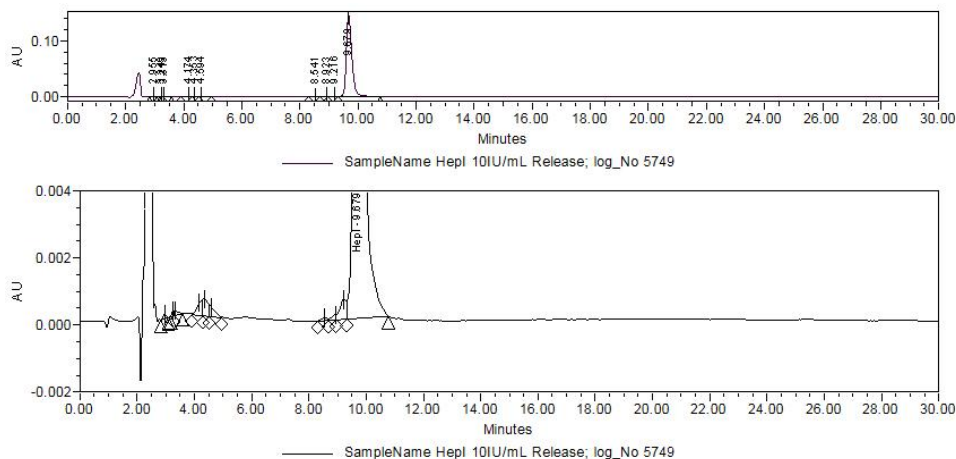
- Heparinase I, produced and supplied by IBEX, is used as a component of the following marketed diagnostic products:
 - HR-HTC High Range Heparinase Test Cartridge (Medtronic Perfusion Systems) for the measurement of activated clotting time (ACT).
 - Heparinase cups and vials (Haemoscope Inc.) for use with the Haemoscope Thromboelastograph® (TEG) Coagulation Analyzer for the monitoring of haemostasis.
 - Hepzyme® (Dade Behring, Inc.) is a lyophilized formulation of Heparinase I for the removal of heparin from freshly drawn blood samples.
 - Abbott (i-STAT) incorporates Heparinase I into its Prothrombin Time test (PT/INR), which is specifically designed for Coumadin® (warfarin) monitoring in patients undergoing oral anticoagulation therapy.
- Neutralization of heparin in blood and plasma samples before analysis (as an additive to or contained in collection tubes).
- As a component of in vitro diagnostic tests for clotting evaluations and platelet function tests for removal of heparin interference.
- Preparation of low molecular weight heparins from unfractionated heparin.
- Research reagent (glycobiology, preparation of oligosaccharide libraries, preparation of disaccharides from heparin).

Potential clinical applications:

- Heparinase I has been developed by IBEX under the commercial name Neutralase™ as an agent for heparin neutralization following cardiovascular bypass surgery. The drug was in Phase III clinical development when it was sold to BioMarin Pharmaceuticals, Novato, California.

Heparinase I Data Sheet

Synonyms	Heparinase; heparin lyase; heparin eliminase
Source	<i>Flavobacterium heparinum</i> (native or recombinant)
EC Number	4.2.2.7
CAS Number	9025-39-2
Catalyzed Reaction	The enzyme cleaves selectively, via an elimination mechanism, highly sulfated polysaccharide chains containing 1-4 linkages between hexosamines and O-sulfated iduronic 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 also cleaves the antithrombin III binding pentasaccharide domain in the heparin molecule.
Substrate Specificity	Heparin; heparan sulfate (specific activity with heparin is approx. 3 times higher than with heparan sulfate).
Properties	<ul style="list-style-type: none"> • Molecular weight: 42,508 Da • Isoelectric point: 9.3 – 9.5 • pH optimum for activity: 6.5 – 7.5 • pH range for activity: 4 – 9 • Optimal temperature range: 20 °C – 37 °C
Purity	≥95 % by reversed phase HPLC analysis.



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Specific Activity

>100 IU/mg using the following Unit definition.

One international unit (IU) is defined as the amount of enzyme that will liberate 1.0 μ mole unsaturated oligosaccharides from porcine mucosal heparin per minute at 30 °C and pH 7.0.

One Unit (U) is also defined in other preparation as 1 U forms 0.1 μ mol of unsaturated uronic acid per hour at 25 °C and pH 7.5; 1 IU is equivalent to 600 U

Stability

- If highly concentrated (≥ 2 mg/ml), several years frozen at -70 °C in aqueous buffers containing Sodium Phosphate or Sucrose 5%.
- PN 50-010 – Expiration is 18 months from manufacturing date frozen at -70 °C in aqueous buffers containing Sodium Phosphate and Sucrose 5%
- PN 50-100 – Expiration is 12 months from manufacturing date frozen at -70 °C in aqueous buffers containing Sodium Phosphate and Sucrose 5%

Applications

- For the neutralization of heparin in blood and plasma samples before analysis.
- For the similar in vitro neutralization of low molecular weight heparins.
- As integral part of in vitro diagnostic tests for the neutralization of heparin (blood clotting tests, platelet tests).
- In blood collection tubes for the neutralization of heparin.
- For the preparation of low molecular weight heparins from unfractionated heparin.
- As a research reagent (glycosaminoglycan degradation).
- For the preparation of disaccharides of heparin 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.
- "Heparinase I from *Flavobacterium heparinum*. Mapping and Characterization of the Heparin Binding Domain". R. Sasisekharan, G. Venkataraman, R. Godavarti, S. Ernst, C.L. Cooney and R. Langer in *J. Biol. Chem.* (1996) 271 (6): 3124-3131.

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- “Cloning and Expression of Heparinase I Gene from *Flavobacterium heparinum*”. R. Sasisekharan, M. Bulmer, K.W. Moremen, C.L. Cooney and R. Langer in *Proc. Natl. Acad. Sci. USA* (1993) 90: 3660-3664.
- “Neutralase (Heparinase I) as a Potential Heparin Reversal Agent in Coronary Artery Bypass Surgery”. P.J. Silver in *Management of Bleeding in Cardiovascular Surgery*, edited by R. Pifarré, MD, (2000) Hanley & Belfus, Inc., Philadelphia, PA.
- “The effects of heparinase I and protamine on platelet reactivity”. T. Ammar and C.F. Fisher in *Anesthesiology* (1997) 86: 1382-1386.
- “Heparinase I (Neutralase) Reversal of Systemic Anticoagulation”. L.G. Michelsen, M. Kikura, J.H. Levy et al. in *Anesthesiology* (1996) 85: 339-346.
- “Neutralase Reverses the Anti-coagulant but not the Anti-thrombotic Activity of Heparin in a Rabbit Model of Venous Thrombosis”. P.J. Silver, R. Broughton, J. Bouthillier et al. in *Thromb. Res.* (1998) 91: 143-150.