Certificate of Analysis

Enoxaparin Sodium Analysis - Enzyme Kit

Cat. No. HEP-ENZ KIT

USP <207> Test for 1,6-Anhydro Derivitive for Enoxaparin Sodium

Intended use	For use in USP <207> Test for 1,6-Anhydro Derivitive for Enoxaparin Sodium and Ph. Eur Enoxaparin Sodium 1097. For depolymerisation of Standard and Test Enoxaparin Sodium solutions. 1 kit provides reagents for 1 sample analysis OR 1 blank and 1 standard solution
Catalogue Number	**HEP-ENZ Kit**
Description	**Enoxaparin Sodium Analysis – Enzyme Kit.**
Kit components	1 Kit contains: **2 x 100ul Heparinase 1, 2, 3 Solution.** 100ul vials of 1: 1: 1 mix of Heparinase 1, Heparinase 2 and Heparinase 3 solutions at a concentration of 0.4IU per ml in Potassium Phosphate buffer pH 7.0 **1 x 200ul Sodium/Calcium Acetate pH 7.0 Solution** 100ul for every vial of enzyme solution. **1 Kit provides Enzyme and Buffer for 1 sample analysis OR 1 bank and 1 standard.**
Batch number	1
Date of preparation	
Expiry	12 months from date of preparation
Storage	-20°C or below

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Heparinase 1, 2, 3 Solution

Product name	Heparinase 1, 2, 3 Solution
Catalogue number	HEP-ENZ 1, 2, 3
Product description	Each vial contains 100µl of a 1:1:1 (v:v:v) mixture of Heparinase 1 solution, Heparinase 2 solution and Heparinase 3 solution. Heparinase 1, Heparinase 2 and Heparinase 3 dissolved in Potassium Phosphate pH 7.0 Buffer (see below) to obtain a concentration of 0.4IU per mL as per USP and Ph. Eur. protocol. The activity of the Heparinase 1, Heparinase 2 and Heparinase 3 stock solutions is determined by the assays described below.
Nature and origin of starting material	*Flavobacterium heparinum* ATCC 13125
Manufacturing process and references	Growth of bacterium: McLean, M.W. et al. (1984) Eur. J. Biochem. 145, 607-615. Purification by further chromatography. Final product 0.22-um sterile filtered and stored at - 60°C.
Impurities	Other enzymes nominally 0.1% max

Heparinase Enzyme Activity Test

Activity (IU/mL) of Heparinase 1, Heparinase 2 and Heparinase 3 stock solutions determined before diluting to 0.4IU/mL and combining into the Heparinase 1, 2, 3 Solution.

One (1) International Unit (IU) defined as **"the amount of enzyme that will liberate 1.0 µmole of product per minute from heparin or heparan sulphate substrate at 30º C" (Product is unsaturated saccharides)**

Heparinase 1 Assay	**Result/Concentration**
Activity determined by increase in absorbance at 232nm. Substrate - commercial porcine heparin at 400ug/mL Buffer - 50mM sodium acetate buffer, pH 7.0 containing 1mM calcium acetate. Enzyme 10mU units Volume 1ml Temp 30°C	1IU in 0.0015mL 667IU/mL
Heparinase 2 Assay	**Result/Concentration**
Activity determined by increase in absorbance at 232nm. Substrate - commercial porcine heparin. 400ug/mL Buffer - 50mM sodium acetate buffer, pH 7.0 containing 1mM calcium acetate. Enzyme 10mU units Volume 1ml Temp 30°C	1IU in 0.0035mL 286IU/mL
Heparinase 3 Assay	**Result/Concentration**
Activity determined by increase in absorbance at 232nm. Substrate - commercial porcine heparan sulphate. 600ug/mL Buffer - 50mM sodium acetate buffer, pH 7.0 containing 1mM calcium acetate. Enzyme 10mU units Volume 1ml Temp 30°C	1IU in 0.0027mL 370IU/mL

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USP <207> Test for 1,6-Anhydro Derivitive for Enoxaparin Sodium

Product name Potassium Phosphate pH 7.0 Buffer

Description Potassium Phosphate pH 7.0 Buffer solution made according to method described in USP <207> Test for 1,6-Anhydro Derivitive for Enoxaparin Sodium and used to make up heparinase enzyme solutions.
Dissolve 10 mg of BSA and 32 mg of calcium acetate in 60mL of water. Add 580 µl of glacial acetic acid, and adjust with 2 M sodium hydroxide toa pH of 7.0. Transfer to a 100mL volumetric flask, and dilute with water to volume. Pass the solution through a filter having a porosity of 0.45 or 0.22 µm.

Test	Method	Specification	Result
pH	Solution made according to method described in USP <207> Test. The buffer is tested using a calibrated pH meter. The buffer is pipetted with a calibrated pipette	pH 7.0	pH 7.0
Appearance	Clear, colourless solution.	Visual inspection	Complies

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USP <207> Test for 1,6-Anhydro Derivitive for Enoxaparin Sodium

Sodium/Calcium acetate pH 7.0 Buffer

Product name Sodium/Calcium acetate pH 7.0 Buffer

Catalogue number BUF 1

Description Sodium/Calcium acetate pH 7.0 buffer solution made according to method described in USP <207> Test for 1,6-Anhydro Derivitive for Enoxaparin Sodium: *Dissolve 10 mg of BSA and 32 mg of calcium acetate in 60mL of water. Add 580 µl of glacial acetic acid, and adjust with 2 M sodium hydroxide toa pH of 7.0. Transfer to a 100mL volumetric flask, and dilute with water to volume. Pass the solution through a filter having a porosity of 0.45 or 0.22 µm.*

The USP protocol requires 70ul of buffer for each test/blank/standard vial.

Test	Method	Specification	Result
pH	Solution made according to method described in USP <207> Test. The buffer is tested using a calibrated pH meter. The buffer is pipetted with a calibrated pipette	pH 7.0	pH 7.0
Appearance	Clear, colourless solution.	Visual inspection	Complies

Approved by:
Prof. J. Gallagher
Iduron CEO

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