

# Certificate of Analysis

## Heparinase I



Batch number	13
Date of manufacture	November 2009
Stability	Heparinase I stable for up to 24 months from the date of delivery when stored at -20°C to -80 °C in a solution of 0.1M Sodium Acetate pH 7.0 containing 1mM Calcium Acetate and 0.1% BSA.
Storage and retest information	Store at -20°C to -80°C and check activity after 24 months.
Nature and origin of starting material	<i>Flavobacterium heparinum</i> 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 deg.C.
Impurities	Other enzymes nominally 0.3% max, other enzymes nominally 0.1% max. Base line resolution from the other two heparinases.
CAS number	9025-39-2
Appearance/form	Supplied as frozen solution containing 0.2% BSA, 0.22um sterile filtered
Specificity	Depolymerises heparin by elimination at the uronic acid. Specific for 2-O-/2-N- sites.
Unit of activity	International units (IU). One international unit is defined as the amount of enzyme that will liberate 1.0 µmole of product per minute from a heparin substrate at 30°C (Product is unsaturated saccharides). Enzyme activity determined using assay below.
Assay	Against commercial porcine heparin in the presence of calcium. Activity determined by absorbance at 232nm.  The unit definition heparinase I is the activity that releases 1 micromole of delta (4, 5) hexuronate per minute at 30°C using an extinction coefficient of 5400 per cm per M at 232nm for the unsaturated (4, 5) hexuronate product.  Assay Conditions: Enzyme buffer: 50mM sodium acetate pH 7.0 with 1mM calcium acetate Substrate: 600ug/ml of heparin equivalent to 1umole of disaccharide Enzyme concentration: 10milliunits/ml Temperature 30C

Approved by:

Prof. J. Gallagher  
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