

Certificate of Analysis

Heparinase III

BN10



Batch number	10
Date of manufacture	November 2009
Stability	Heparinase III 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 0.1% max. Base line resolution from the other two heparinases.
CAS number	37290-86-1
Appearance/form	Supplied as frozen solution containing 0.2% BSA, 0.22um sterile filtered
Specificity	Depolymerises heparan sulphate by elimination at the uronic acid. Acts next to N-sulphate or N-acetate, in sites with low O-sulphation. However some of these sites are totally resistant to Hep III (and also Hep II).
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 or dermatan sulphate substrate at 25° C" (Product is unsaturated saccharides). Enzyme activity determined using assay below.
Assay	<p>Against commercial porcine heparin in the presence of calcium. Activity determined by absorbance at 232nm.</p> <p>The unit definition heparinase III 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.</p> <p>Assay Conditions: Enzyme buffer: 50mM sodium acetate pH 7.0 with 1mM calcium acetate Substrate: 500ug/ml of heparin Sulphate equivalent to 1umole of disaccharide Enzyme concentration: 10milliunits/ml Temperature 30C</p>

Approved by:

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