

# Certificate of Analysis

## Heparinase III

### BN11



Batch number	11
Date of manufacture	January 2016
Stability	Heparinase III stable for up to 24 months from the date of delivery when stored at -20°C or below in a solution of 0.1M Sodium Acetate pH 7.0 containing 1mM Calcium Acetate and 0.1% BSA.
Storage	Store at -20°C or below
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.
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 0.3% 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 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 heparan sulphate substrate at 30° C" (Product is unsaturated saccharides). Enzyme activity determined using assay below.
Assay	Against commercial porcine heparan sulphate. Activity determined by absorbance at 232nm.  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.
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 30°C
Approved by: Prof. J. Gallagher Iduron CEO	