

New England Biolabs Product Specification

Product Name:	<i>Bacteroides Heparinase III</i>
Catalog #:	P0737S/L
Concentration:	700 units/ml
Unit Definition:	One unit is defined as the amount of enzyme that will liberate 1.0 μmol unsaturated oligosaccharides from heparan sulfate per minute at 30°C and pH 7.0 in a total reaction volume of 100 μl .
Shelf Life:	12 months
Storage Temp:	-80°C
Storage Conditions:	100 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, 5 mM CaCl ₂ , (pH 7.5 @ 25°C)
Specification Version:	PS-P0737S/L v1.0
Effective Date:	09 Dec 2015

Assay Name/Specification (minimum release criteria)

Glycosidase Activity (β 1-3 Galactosidase) - A 10 μl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC) and 1 unit of *Bacteroides Heparinase III* incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (β 1-4 Galactosidase) - A 10 μl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC) and 1 unit of *Bacteroides Heparinase III* incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (β -N-Acetylgalactosaminidase) - A 10 μl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled β -N-Acetylgalactosaminidase substrate (GalNAc β 1-4Gal β 1-4Glc-AMC) and 1 unit of *Bacteroides Heparinase III* incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (β -N-Acetylglucosaminidase) - A 10 μl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled β -N-Acetylglucosaminidase substrate (GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC) and 1 unit of *Bacteroides Heparinase III* incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.

Protease Activity (SDS-PAGE) - A 20 μl reaction in 1X Heparinase Reaction Buffer containing 24 μg of a standard mixture of proteins and a minimum of 5 units of *Bacteroides Heparinase III* incubated for 20 hours at 37°C, results in no detectable degradation of the protein mixture as determined by SDS-PAGE with Coomassie Blue detection.

Protein Purity Assay (SDS-PAGE) - *Bacteroides Heparinase III* is $\geq 95\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



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Assay Name/Specification (minimum release criteria)

<p>Sulfatase Activity (2-O) - A 10 μl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled 2-O-Sulfatase substrate (ΔUA2S-(1-4)-GlcNS6S-AMC) and 1 unit of <i>Bacteroides</i> Heparinase III incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.</p>
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<p>Sulfatase and Uronidase Activity (N,6-O) - A 10 μl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled N,6-O-Sulfatase substrate (ΔUA-(1-4)-GlcNS6S-AMC) and 1 unit of <i>Bacteroides</i> Heparinase III incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.</p>
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Date 09 Dec 2015

Derek Robinson
Director of Quality Control

