

## New England Biolabs Certificate of Analysis

*Product Name:* *Bacteroides Heparinase III*  
*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.*  
*Lot #:* *0031610*  
*Assay Date:* *10/2016*  
*Expiration Date:* *10/2017*  
*Storage Temp:* *-80°C*  
*Storage Conditions:* *100 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, 5 mM CaCl<sub>2</sub>, (pH 7.5 @ 25°C)*  
*Specification Version:* *PS-P0737S/L v1.0*  
*Effective Date:* *19 Feb 2016*

Assay Name/Specification (minimum release criteria)	Lot #0031610
<b>Glycosidase Activity (β1-3 Galactosidase)</b> - A 10 μl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-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.	<b>Pass</b>
<b>Glycosidase Activity (β1-4 Galactosidase)</b> - A 10 μl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -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.	<b>Pass</b>
<b>Glycosidase Activity (β-N-Acetylgalactosaminidase)</b> - A 10 μl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-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.	<b>Pass</b>
<b>Glycosidase Activity (β-N-Acetylglucosaminidase)</b> - A 10 μl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled β-N-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-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.	<b>Pass</b>



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Assay Name/Specification (minimum release criteria)	Lot #0031610
<b>Protease Activity (SDS-PAGE)</b> - 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 <i>Bacteroides</i> 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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - <i>Bacteroides</i> Heparinase III is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>Sulfatase Activity (2γ)</b> - 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.	<b>Pass</b>
<b>Sulfatase and Uronidase Activity (N,6γ)</b> - 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.	<b>Pass</b>



Authorized by  
Derek Robinson  
19 Feb 2016



Inspected by  
Alicia Bielik  
25 Oct 2016

