

## New England Biolabs Certificate of Analysis

**Product Name:** *Bacteroides Heparinase III*  
**Catalog Number:** *P0737L*  
**Concentration:** *700 U/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.*  
**Packaging Lot Number:** *10070625*  
**Expiration Date:** *08/2021*  
**Storage Temperature:** *-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*

Bacteroides Heparinase III Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
P0737LVIAL	Bacteroides Heparinase III	10078228	Pass
B0735SVIAL	Bacteroides Heparinase Reaction Buffer (10X)	10081039	Pass

Assay Name/Specification	Lot # 10070625
<p><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 Bacteroides Heparinase III incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><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 Bacteroides Heparinase III incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><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 Bacteroides Heparinase III incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass

Assay Name/Specification	Lot # 10070625
<p><b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b> A 10 <math>\mu</math>l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled <math>\beta</math>-N-Acetylglucosaminidase substrate (GlcNAc<math>\beta</math>1-4GlcNAc<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Protease Activity (SDS-PAGE)</b> A 20 <math>\mu</math>l reaction in 1X Heparinase Reaction Buffer containing 24 <math>\mu</math>g 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.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> Bacteroides Heparinase III is <math>\geq</math> 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>Sulfatase Activity (2-O)</b> A 10 <math>\mu</math>l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled 2-O-Sulfatase substrate (<math>\Delta</math>UA2S-(1-4)-GlcNS6S-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.</p>	<b>Pass</b>
<p><b>Sulfatase and Uronidase Activity (N,6-O)</b> A 10 <math>\mu</math>l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled N,6-O-Sulfatase substrate (<math>\Delta</math>UA-(1-4)-GlcNS6S-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.</p>	<b>Pass</b>

This product has been tested and shown to be in compliance with all specifications.

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*Alicia Bielik*

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Alicia Bielik  
Production Scientist  
23 Sep 2020

*Michael Tonello*

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Michael Tonello  
Packaging Quality Control Inspector  
23 Sep 2020