

## New England Biolabs Certificate of Analysis

**Product Name:** *Bacteroides Heparinase I*  
**Catalog #:** P0735S/L  
**Concentration:** 12,000 units/ml  
**Unit Definition:** One unit is defined as the amount of enzyme that will liberate 1.0 μmol unsaturated oligosaccharides from porcine mucosal heparin per minute at 30°C and pH 7.0 in a total reaction volume of 100 μl.  
**Lot #:** 0031703  
**Assay Date:** 03/2017  
**Expiration Date:** 03/2018  
**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-P0735S/L v1.0  
**Effective Date:** 16 Feb 2016

Assay Name/Specification (minimum release criteria)	Lot #0031703
<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 24 units of <i>Bacteroides</i> Heparinase I 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 24 units of <i>Bacteroides</i> Heparinase I 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 24 units of <i>Bacteroides</i> Heparinase I 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 24 units of <i>Bacteroides</i> Heparinase I 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 #0031703
<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 120 units of <i>Bacteroides</i> Heparinase I 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 I 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 24 units of <i>Bacteroides</i> Heparinase I 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 24 units of <i>Bacteroides</i> Heparinase I 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  
16 Feb 2016



Inspected by  
Alicia Bielik  
08 Mar 2017

