240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## 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  $\mu$ l.

 Lot #:
 0031801

 Assay Date:
 01/2018

 Expiration Date:
 1/2019

 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 #0031801
Glycosidase Activity ( $\beta$ 1-3 Galactosidase) - A 10 $\mu$ l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 24 units of Bacteroides Heparinase I incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\beta$ 1-4 Galactosidase) - A 10 $\mu$ l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc -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.	Pass
Glycosidase Activity (β-N-Acetylgalactosaminidase) - 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.	Pass
Glycosidase Activity ( $\beta$ -N-Acetylglucosaminidase) - A 10 $\mu$ l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-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.	Pass









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Assay Name/Specification (minimum release criteria)	Lot #0031801
Protease Activity (SDS-PAGE) - A 20 $\mu$ l reaction in 1X Heparinase Reaction Buffer containing 24 $\mu$ g 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.	Pass
<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.	Pass
<b>Sulfatase Activity (2γ)</b> - A 10 μl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled 2- <i>O</i> -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.	Pass
<b>Sulfatase and Uronidase Activity (N,6y)</b> - A 10 $\mu$ l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled N,6- $O$ -Sulfatase substrate ( $\Delta$ UA-(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.	Pass

Authorized by Derek Robinson 16 Feb 2016







Inspected by Brad Landgraf 23 Jan 2018