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 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 μ l.

 Lot #:
 0031708

 Assay Date:
 08/2017

 Expiration Date:
 08/2018

 Storage Temp:
 -80°C

Storage Conditions: 100 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, 5 mM CaCl₂, (pH 7.5 @ 25°C)

Specification Version: PS-P0737S/L v1.0 Effective Date: 19 Feb 2016

| Assay Name/Specification (minimum release criteria) | Lot #0031708 |
|--|--------------|
| Glycosidase Activity (β 1-3 Galactosidase) - A 10 μ l 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. | Pass |
| Glycosidase Activity (β 1-4 Galactosidase) - A 10 μ l 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 <i>Bacteroides</i> Heparinase III 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 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. | Pass |
| Glycosidase Activity (β -N-Acetylglucosaminidase) - A 10 μ l 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 <i>Bacteroides</i> Heparinase III 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 #0031708 |
|---|--------------|
| Protease Activity (SDS-PAGE) - A 20 μ l reaction in 1X Heparinase Reaction Buffer containing 24 μ g 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. | Pass |
| Protein Purity Assay (SDS-PAGE) - <i>Bacteroides</i> Heparinase III is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |
| Sulfatase Activity (2γ) - 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 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. | Pass |
| Sulfatase and Uronidase Activity (N,6y) - A 10 μ l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled N,6- O -Sulfatase substrate (Δ UA-(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. | Pass |

Authorized by Derek Robinson 19 Feb 2016







Inspected by Brad Landgraf 09 Aug 2017