

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 #: *0031608*
Assay Date: *08/2016*
Expiration Date: *8/2017*
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 #0031608 |
|---|--------------|
| Glycosidase Activity (β1-3 Galactosidase) - 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. | Pass |
| Glycosidase Activity (β1-4 Galactosidase) - 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. | 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 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. | Pass |



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| Protease Activity (SDS-PAGE) - 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. | 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-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. | Pass |
| Sulfatase and Uronidase Activity (N,6γ) - 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. | Pass |



Authorized by
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19 Feb 2016



Inspected by
Alicia Bielik
02 Aug 2016

