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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 Number:	P0737S
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:	10182193
Expiration Date:	02/2024
Storage Temperature:	-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

Bacteroides Heparinase III Component List			
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result
P0737SVIAL	Bacteroides Heparinase III	10180326	Pass
B0735SVIAL	Bacteroides Heparinase Reaction Buffer (10X)	10181104	Pass

Assay Name/Specification	Lot # 10182193
<b>Glycosidase Activity (<math>\beta</math>-N-Acetylgalactosaminidase)</b> A 10 µl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 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
<b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b> A 10 µl reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 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.	Pass
<b>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b> A 10 µl 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 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





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Assay Name/Specification	Lot # 10182193
<b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b> A 10 µl 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 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
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 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.	Pass
Protein Purity Assay (SDS-PAGE) Bacteroides Heparinase III is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
Sulfatase Activity (2-O) A 10 $\mu$ I reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled 2-O-Sulfatase substrate ( $\Delta$ 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.	Pass
Sulfatase and Uronidase Activity (N,6-O) A 10 $\mu$ I reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled N,6-O-Sulfatase substrate ( $\Delta$ 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.	Pass

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

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