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: α1-6 Mannosidase

Catalog #: P0727S/L
Concentration: 40,000 units/ml

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α -D-mannose from 1 nmol of Man α 1-

6Man α 1-6Man-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37 $^{\circ}$ C in a total reaction volume of 10 μ l.

 Lot #:
 0091609

 Assay Date:
 09/2016

 Expiration Date:
 9/2017

 Storage Temp:
 4°C

Storage Conditions: 50 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, (pH 7.5 @ 25°C)

Specification Version: PS-P0727S/L v1.0 Effective Date: 19 Feb 2016

Assay Name/Specification (minimum release criteria)	Lot #0091609
Glycosidase Activity (Endo F1, F2, H) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (Endo F2, F3) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (PNGase F) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β-Mannosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β-Xylosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass









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Glycosidase Activity (β 1-3 Galactosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°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 Glyco Buffer 1 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc -AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β -N-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β -N-Acetylgalactosaminidase substrate (GalNAc β 1-4Gal β 1-4Glc-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β -N-Acetylglucosaminidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β -N-Acetylglucosaminidase substrate (GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α -Glucosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Glucosidase substrate (Glc α 1-6Glc α 1-4Glc-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α -Neuraminidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Neuraminidase substrate (Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α 1-2 Fucosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc α 1-2Gal β 1-4Glc-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α 1-3 Fucosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc α 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass









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Assay Name/Specification (minimum release criteria)	Lot #0091609
Glycosidase Activity (α 1-3 Galactosidase) - A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-3Gal β 1-4GlcNAc-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α1-3 Mannosidase) - A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 80 units of α1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α 1-6 Galactosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α -N-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -N-Acetylgalactosaminidase substrate (GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC) and 80 units of α 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Protease Activity (SDS-PAGE) - A 20 μ l reaction in 1X Glyco Buffer 1 containing 24 μ g of a standard mixture of proteins and a minimum of 400 units of α 1-6 Mannosidase 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) - $\alpha 1$ -6 Mannosidase is $\geq 95\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass

Authorized by Derek Robinson 19 Feb 2016







Inspected by Alicia Bielik 04 Oct 2016