

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 Number: P0727S
Concentration: 40,000 U/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

Manα1-6Manα1-6Man-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C

in a total reaction volume of 10  $\mu$ l.

Packaging Lot Number: 10082244
Expiration Date: 08/2021
Storage Temperature: 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

α1-6 Mannosidase Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
P0727SVIAL	α1-6 Mannosidase	10082245	Pass	
B9001SVIAL	Purified BSA	10059427	Pass	
B1727SVIAL	10X GlycoBuffer 1	10066905	Pass	

Assay Name/Specification	Lot # 10082244
Glycosidase Activity (Endo F1, F2, H) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) 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
Protein Purity Assay (SDS-PAGE) α1-6 Mannosidase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
Glycosidase Activity (Endo F2, F3) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) 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 (PNGase F)	Pass



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Assay Name/Specification	Lot # 10082244
A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 80 units of $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	
Glycosidase Activity (α-Glucosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-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-N-Acetylgalactosaminidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fucα1-2)Galβ1-4Glc-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-Neuraminidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-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 (α1-2 Fucosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-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 (α1-3 Fucosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-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 (α1-3 Galactosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-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 (α1-3 Mannosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled	Pass



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Assay Name/Specification	Lot # 10082244
$\alpha\text{-Mannosidase}$ substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC) and 80 units of $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	
Glycosidase Activity ( $\alpha$ 1-6 Galactosidase) A 10 $\mu$ I reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 80 units of $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (β-Mannosidase)</b> A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC) and 80 units of $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (β-N-Acetylgalactosaminidase)</b> A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-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 (β-N-Acetylglucosaminidase) A 10 $\mu$ I reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-N-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-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 (β-Xylosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-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 (β1-3 Galactosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-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 (β1-4 Galactosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 80 units of α1-6	Pass



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Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	
Protease Activity (SDS-PAGE) A 20 μl reaction in 1X Glyco Buffer 1 containing 24 μg of a standard mixture of proteins and a minimum of 400 units of α1-6 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

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

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Alicia Bielik Production Scientist 25 Aug 2020 Michael Tonello

Packaging Quality Control Inspector

25 Aug 2020



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