NEW ENGLAND BioLabs Inc.

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New England Biolabs
Certificate of Analysis

Product Name:	*α1-2,3 Mannosidase*
Catalog #:	*P0729S/L*
Concentration:	*32,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to cleave > 95% of the non-reducing terminal α-D-mannose from 1 nmol Manα1-3Manβ1-4GlcNAc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.*
Lot #:	*0191804*
Assay Date:	*04/2018*
Expiration Date:	*4/2019*
Storage Temp:	*4°C*
Storage Conditions:	*50 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, (pH 7.5 @ 25°C)*
Specification Version:	*PS-P0729S/L v1.0*
Effective Date:	*19 Feb 2016*

Assay Name/Specification (minimum release criteria)	**Lot #0191804**
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 32 units of α1-2,3 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 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 32 units of α1-2,3 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 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 32 units of α1-2,3 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 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 32 units of α1-2,3 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 32 units of α1-2,3 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 #0191804**
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 32 units of α1-2,3 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 32 units of α1-2,3 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 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-*N*-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-AMC) and 32 units of α1-2,3 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 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-*N*-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC) and 32 units of α1-2,3 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 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 32 units of α1-2,3 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 32 units of α1-2,3 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 32 units of α1-2,3 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 32 units of α1-2,3 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 #0191804**
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 32 units of α1-2,3 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (α1-6 Galactosidase) - A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-AMC) and 32 units of α1-2,3 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 32 units of α1-2,3 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 µl reaction in 1X Glyco Buffer 1 containing 24 µg of a standard mixture of proteins and a minimum of 400 units of α1-2,3 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) - α1-2,3 Mannosidase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**

Authorized by
Derek Robinson
19 Feb 2016

Inspected by
Alicia Bielik
24 Apr 2018

nqa ISO 9001 Registered Quality Management
nqa ISO 14001 Registered Environmental Management
nqa ISO 13485 Registered Medical Devices