NEW ENGLAND BioLabs Inc.

240 County Road
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New England Biolabs
Certificate of Analysis

Product Name:	*O-Glycosidase*
Catalog #:	*P0733S/L*
Concentration:	*40,000,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to remove 0.68 nmol of O-linked disaccharide from 5 mg of neuraminidase digested, non-denatured fetuin in 1 hour at 37°C in a total reaction volume of 100 µl (1 unit of both O-Glycosidase and PNGase F will remove equivalent molar amounts of O-linked disaccharides and N-linked oligosaccharides, respectively).*
Lot #:	*0031603*
Assay Date:	*03/2016*
Expiration Date:	*3/2018*
Storage Temp:	*-20°C*
Storage Conditions:	*50 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, (pH 7.5 @ 25°C)*
Specification Version:	*PS-P0733S/L v1.0*
Effective Date:	*16 Feb 2016*

Assay Name/Specification (minimum release criteria)	**Lot #0031603**
Glycosidase Activity (Endo F1, F2, H) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled β-*N*-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-AMC) and 200,000 units of *O*-Glycosidase 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-N-Acetylglucosaminidase) - A 10 αl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-*N*-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled α-*N*-Acetylgalactosaminidase substrate (GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-AMC) and 200,000 units of *O*-Glycosidase 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 (α1-3 Fucosidase) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-AMC) and 200,000 units of *O*-Glycosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (α1-6 Mannosidase) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-6Manα1-6(Manα1-3)Man-AMC) and 200,000 units of *O*-Glycosidase 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 2 containing 24 µg of a standard mixture of proteins and a minimum of 1,000,000 units of *O*-Glycosidase 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) - *O*-Glycosidase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**

Authorized by
Derek Robinson
16 Feb 2016

Inspected by
Alicia Bielik
05 Apr 2016

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