

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:	O-Glycosidase
Catalog Number:	P0733S
Concentration:	40,000,000 U/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 $\mu$ l (1 unit of both O-Glycosidase and PNGase F will remove equivalent molar amounts of O-linked disaccharides and N-linked oligosaccharides, respectively).
Packaging Lot Number:	10149065
Expiration Date:	01/2024
Storage Temperature:	-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

O-Glycosidase Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
P0733SVIAL	O-Glycosidase	10137158	Pass	
B3704SVIAL	10X GlycoBuffer 2	10118382	Pass	
B2704SVIAL	NP-40	10129282	Pass	
B1704SVIAL	Glycoprotein Denaturing Buffer	10119058	Pass	

Assay Name/Specification	Lot # 10149065
<b>Glycosidase Activity (PNGase F)</b> 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
<b>Glycosidase Activity (Endo F2, F3)</b> 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
<b>Glycosidase Activity (Endo F1, F2, H)</b> A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled Endo F1,	Pass





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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.	
<b>Protease Activity (SDS-PAGE)</b> 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
<b>Glycosidase Activity (β-Xylosidase)</b> 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
<b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b> A 10 ·I reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-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
<b>Glycosidase Activity (<math>\beta</math>-N-Acetylgalactosaminidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-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
<b>Glycosidase Activity (β-Mannosidase)</b> 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
<b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-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





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<b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-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
<b>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b> A 10 $\mu$ I reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-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
<b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)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
<b>Glycosidase Activity (α1-3 Fucosidase)</b> 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
<b>Glycosidase Activity (<math>\alpha</math>1-2 Fucosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-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
<b>Glycosidase Activity (α1-3 Galactosidase)</b> 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
<b>Glycosidase Activity (<math>\alpha</math>-Neuraminidase)</b> A 10 $\mu$ I reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Neuraminidase substrate (Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-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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<b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc -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
<b>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-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
<b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-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

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

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Alicia Bielik Production Scientist 28 Apr 2022

Erin Varney

Packaging Quality Control Inspector 28 Apr 2022

