

New England Biolabs Certificate of Analysis

Product Name: O-Glycosidase
Catalog Number: P0733L
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 µl (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: 10128264
Expiration Date: 11/2023
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
P0733LVIAL	O-Glycosidase	10128265	Pass
B3704SVIAL	10X GlycoBuffer 2	10118382	Pass
B2704SVIAL	NP-40	10098512	Pass
B1704SVIAL	Glycoprotein Denaturing Buffer	10119058	Pass

Assay Name/Specification	Lot # 10128264
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 (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 (α1-6 Mannosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled	Pass

Assay Name/Specification	Lot # 10128264
<p>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.</p>	
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>Glycosidase Activity (β-N-Acetylglucosaminidase) A 10 μl 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.</p>	Pass
<p>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.</p>	Pass
<p>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</p>	Pass

Assay Name/Specification	Lot # 10128264
<p>O-Glycosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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</p>	Pass

Assay Name/Specification	Lot # 10128264
thin layer chromatography.	
<p>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.</p>	Pass
<p>Protein Purity Assay (SDS-PAGE) O-Glycosidase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>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.</p>	Pass

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.



Alicia Bielik
Production Scientist
18 Nov 2021



Michael Tonello
Packaging Quality Control Inspector
18 Nov 2021