

## 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:** 10106703  
**Expiration Date:** 04/2023  
**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	10106704	Pass
B3704SVIAL	10X GlycoBuffer 2	10091370	Pass
B2704SVIAL	NP-40	10098512	Pass
B1704SVIAL	Glycoprotein Denaturing Buffer	10090539	Pass

Assay Name/Specification	Lot # 10106703
<b>Protein Purity Assay (SDS-PAGE)</b> O-Glycosidase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<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
<b>Glycosidase Activity (α1-3 Mannosidase)</b> 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	Pass

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determined by thin layer chromatography.	
<p><b>Glycosidase Activity (<math>\alpha</math>1-3 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Galactosidase substrate (Gal<math>\alpha</math>1-3Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Galactosidase substrate (Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Galactosidase substrate (Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-4Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>-N-Acetylgalactosaminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\beta</math>1-4Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Mannosidase substrate (Man<math>\beta</math>1-4Man<math>\beta</math>1-4Man-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>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Mannosidase substrate (Man<math>\alpha</math>1-6Man<math>\alpha</math>1-6(Man<math>\alpha</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Galactosidase substrate (Gal<math>\alpha</math>1-6Gal<math>\alpha</math>1-6Glc<math>\alpha</math>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.</p>	<b>Pass</b>

Assay Name/Specification	Lot # 10106703
<p><b>Glycosidase Activity (<math>\beta</math>-Xylosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Xylosidase substrate (Xyl<math>\beta</math>1-4Xyl<math>\beta</math>1-4Xyl<math>\beta</math>1-4Xyl-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>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-N-Acetylglucosaminidase substrate (GlcNAc<math>\beta</math>1-4GlcNAc<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Glucosidase substrate (Glc<math>\alpha</math>1-6Glc<math>\alpha</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\alpha</math>1-3(Fuca<math>\alpha</math>1-2)Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-2 Fucosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuca<math>\alpha</math>1-2Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>-Neuraminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Neuraminidase substrate (Neu5Ac<math>\alpha</math>2-3Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-3 Fucosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuca<math>\alpha</math>1-3Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>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.</p>	<b>Pass</b>

Assay Name/Specification	Lot # 10106703
<p><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.</p>	<b>Pass</b>
<p><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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (Endo F1, F2, H)</b> 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>	<b>Pass</b>

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

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21 May 2021



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