

## 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 #:* 0031612  
*Assay Date:* 12/2016  
*Expiration Date:* 12/2018  
*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 #0031612
<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.	<b>Pass</b>
<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.	<b>Pass</b>
<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.	<b>Pass</b>
<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.	<b>Pass</b>
<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.	<b>Pass</b>

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<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 <i>O</i>-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 <i>O</i>-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 <i>O</i>-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 <i>O</i>-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 <i>O</i>-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 <i>O</i>-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 (Fuc<math>\alpha</math>1-2Gal<math>\beta</math>1-4Glc-AMC) and 200,000 units of <i>O</i>-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 (Fuc<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 <i>O</i>-Glycosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>

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Assay Name/Specification (minimum release criteria)	Lot #0031612
<b>Glycosidase Activity (<math>\alpha</math>1-3 Galactosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> - A 10 $\mu$ l 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> - A 10 $\mu$ l 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b> - A 10 $\mu$ l 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> - A 10 $\mu$ l 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.	<b>Pass</b>
<b>Protease Activity (SDS-PAGE)</b> - A 20 $\mu$ l reaction in 1X Glyco Buffer 2 containing 24 $\mu$ g 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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - O-Glycosidase is $\geq$ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



Authorized by  
Derek Robinson  
16 Feb 2016



Inspected by  
Alicia Bielik  
19 Dec 2016

