

## New England Biolabs Certificate of Analysis

**Product Name:** O-Glycoprotease  
**Catalog Number:** P0761S  
**Concentration:** 1,000 U/ml  
**Unit Definition:** One unit of O-Glycoprotease will cleave >90% of 2  $\mu$ M FAM-labeled O-glycopeptide in a total reaction volume of 20  $\mu$ l in 2 hours at 37°C in 20mM Tris-HCl, pH 8.0.  
**Packaging Lot Number:** 10140322  
**Expiration Date:** 03/2024  
**Storage Temperature:** -20°C  
**Storage Conditions:** 20 mM Tris-HCl, 100 mM NaCl (pH 7.5 @ 25°C)  
**Specification Version:** PS-P0761S v1.0

O-Glycoprotease Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
P0761SVIAL	O-Glycoprotease	10140321	Pass

Assay Name/Specification	Lot # 10140322
<b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> A 10 $\mu$ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 2 units of O-Glycoprotease 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>1-3 Mannosidase)</b> A 10 $\mu$ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC) and 2 units of O-Glycoprotease 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>1-3 Galactosidase)</b> A 10 $\mu$ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-AMC) and 2 units of O-Glycoprotease 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>1-3 Fucosidase)</b> A 10 $\mu$ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of	Pass

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<p>fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuca1-3Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>1-4Glc-AMC) and 2 units of O-Glycoprotease incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	
<p><b>Glycosidase Activity (<math>\alpha</math>1-2 Fucosidase)</b> A 10 <math>\mu</math>l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuca1-2Gal<math>\beta</math>1-4Glc-AMC) and 2 units of O-Glycoprotease 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 <math>\mu</math>l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 2 units of O-Glycoprotease 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 F2, F3)</b> A 10 <math>\mu</math>l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 2 units of O-Glycoprotease 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 <math>\mu</math>l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 2 units of O-Glycoprotease incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Protease Activity (Non-Specific, SDS-PAGE)</b> A 20 <math>\mu</math>l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 24 <math>\mu</math>g of a standard mixture of proteins and a minimum of 5 units of O-Glycoprotease was incubated for 20 hours at 37°C. After incubation, no detectable degradation of the protein mixture was determined by SDS-PAGE with Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b> A 10 <math>\mu</math>l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) 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 2 units of O-Glycoprotease 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 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of</p>	<b>Pass</b>

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<p>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 2 units of O-Glycoprotease incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	
<p><b>Glycosidase Activity (<math>\beta</math>-Xylosidase)</b> A 10 <math>\mu</math>l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) 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 2 units of O-Glycoprotease 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 20 mM Tris-HCl (pH 8.0 @ 25°C) 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 2 units of O-Glycoprotease 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 20 mM Tris-HCl (pH 8.0 @ 25°C) 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 2 units of O-Glycoprotease 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 20 mM Tris-HCl (pH 8.0 @ 25°C) 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 2 units of O-Glycoprotease 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 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled <math>\beta</math>-Mannosidase substrate (Man<math>\beta</math>1-4Man<math>\beta</math>1-4Man-AMC) and 2 units of O-Glycoprotease 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 20 mM Tris-HCl (pH 8.0 @ 25°C) 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 2 units of O-Glycoprotease 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></p>	<b>Pass</b>

Assay Name/Specification	Lot # 10140322
<p>A 10 µl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fuca1-2)Galβ1-4Glc-AMC) and 2 units of O-Glycoprotease incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p> <p><b>Glycosidase Activity (α-Glucosidase)</b> A 10 ul reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 2 units of O-Glycoprotease incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<p><b>Pass</b></p>

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

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17 Mar 2022



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