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## New England Biolabs Certificate of Analysis

Product Name: O-Glycoprotease (IMPa)

Catalog Number: P0761S
Concentration: 1,000 U/ml

Unit Definition: One unit of O-Glycoprotease will cleave >90% of 2 μM FAM-labeled

O-glycopeptide in a total reaction volume of 20 µl in 2 hours at

37°C in 20mM Tris-HCl, pH 8.0.

Packaging Lot Number: 10207546
Expiration Date: 09/2025
Storage Temperature: -20°C

Storage Conditions: 20 mM Tris-HCl, 100 mM NaCl (pH 7.5 @ 25°C)

Specification Version: PS-P0761S v2.0

O-Glycoprotease (IMPa) Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
P0761SVIAL	O-Glycoprotease (IMPa)	10201204	Pass	

Assay Name/Specification	Lot # 10207546
Glycosidase Activity (Endo F1, F2, H) A 10 μl 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 (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (Endo F2, F3) A 10 µl 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 (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (PNGase F) A 10 µl 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 (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α-Glucosidase) A 10 ul reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of	Pass



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Assay Name/Specification	Lot # 10207546
fluorescently-labeled $\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	
Glycosidase Activity (α-N-Acetylgalactosaminidase) 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(Fucα1-2)Galβ1-4Glc-AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α-Neuraminidase) A 10 μl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at $37^{\circ}$ C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\alpha$ 1-2 Fucosidase) A 10 $\mu$ I reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC) and 2 units of O-Glycoprotease (IMPa) 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 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at $37^{\circ}$ C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\alpha$ 1-3 Galactosidase) A 10 $\mu$ I reaction in 20 mM Tris-HCI (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 (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (α1-3 Mannosidase)</b> A 10 μl reaction in 20 mM Tris-HCl (pH $8.0 \ @ 25^{\circ}$ C) containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass



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Assay Name/Specification	Lot # 10207546
Glycosidase Activity (α1-6 Galactosidase) A 10 μl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-AMC) and 2 units of O-Glycoprotease (IMPa) 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 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-6(Manα1-3)Man-AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β-Mannosidase) A 10 μl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β-N-Acetylgalactosaminidase) A 10 μl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β-N-Acetylglucosaminidase) A 10 μl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β-N-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β-Xylosidase) A 10 μl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β1-3 Galactosidase) A 10 μl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass



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Assay Name/Specification	Lot # 10207546
Glycosidase Activity (β1-4 Galactosidase) A 10 μl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 2 units of O-Glycoprotease (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Protease Activity (Non-Specific, SDS-PAGE) A 20 µl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 24 µg of a standard mixture of proteins and a minimum of 5 units of O-Glycoprotease (IMPa) was incubated	Pass

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

for 20 hours at 37°C. After incubation, no detectable degradation of the protein mixture was determined by SDS-PAGE with Coomassie Blue detection.

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Maxwell/Elkus Production Scientist 25 Aug 2023 Michael Tonello

Packaging Quality Control Inspector

11 Sep 2023



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