

New England Biolabs Product Specification

Product Name:	<i>O-Glycoprotease (IMPa)</i>
Catalog #:	P0761S
Concentration:	1,000 units/ml
Unit Definition:	One unit of <i>O-Glycoprotease</i> will cleave >90% of 2 μ M FAM-labeled <i>O-glycopeptide</i> in a total reaction volume of 20 μ l in 2 hours at 37°C in 20mM Tris-HCl, pH 8.0.
Shelf Life:	24 months
Storage Temp:	-20°C
Storage Conditions:	20 mM Tris-HCl, 100 mM NaCl (pH 7.5 @ 25°C)
Specification Version:	PS-P0761S v2.0
Effective Date:	27 Sep 2022

Assay Name/Specification (minimum release criteria)

Glycosidase Activity (Endo F1, F2, H) - A 10 μ 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* (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (Endo F2, F3) - A 10 μ 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* (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (PNGase F) - A 10 μ 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* (IMPa) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (α -Glucosidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α -Glucosidase substrate (Glc α 1-6Glc α 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 -Neuraminidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α -Neuraminidase substrate (Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 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 1-2 Fucosidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc α 1-2Gal β 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.



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Glycosidase Activity (α 1-3 Fucosidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuca1-3Gal β 1-4GlcNAc β 1-3Gal β 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 1-3 Galactosidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-3Gal β 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.

Glycosidase Activity (α 1-3 Mannosidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-3Man β 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.

Glycosidase Activity (α 1-6 Galactosidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-6Gal α 1-6Glc α 1-2Fru-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.

Glycosidase Activity (α 1-6 Mannosidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-6Man α 1-6(Man α 1-3)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.

Glycosidase Activity (α -*N*-Acetylgalactosaminidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α -*N*-Acetylgalactosaminidase substrate (GalNAc α 1-3(Fuca1-2)Gal β 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 (β -Mannosidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β -Mannosidase substrate (Man β 1-4Man β 1-4Man-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.

Glycosidase Activity (β -Xylosidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β -Xylosidase substrate (Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-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.

Glycosidase Activity (β 1-3 Galactosidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-3GlcNAc β 1-4Gal β 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 (β 1-4 Galactosidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-4GlcNAc β 1-3Gal β 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.



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Glycosidase Activity (β -N-Acetylgalactosaminidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β -N-Acetylgalactosaminidase substrate (GalNAc β 1-4Gal β 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 (β -N-Acetylglucosaminidase) - A 10 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled β -N-Acetylglucosaminidase substrate (GlcNAc β 1-4GlcNAc β 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.

Protease Activity (Non-Specific, SDS-PAGE) - A 20 μ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 24 μ g of a standard mixture of proteins and a minimum of 5 units of O-Glycoprotease (IMPa) 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.

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Lauren Brown
Quality Approver

