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New England Biolabs
Product Specification

Product Name:	*O-Glycoprotease (IMPa)*
Catalog #:	*P0761S*
Concentration:	*1,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to cleave >90% of 2 pmol FAM-labeled O-glycopeptide in 2 hours at 37°C in a total reaction volume of 20 µl.*
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 v3.0*
Effective Date:	*17 Oct 2024*

Assay Name/Specification (minimum release criteria)
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.
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.
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.
Glycosidase Activity (α-Glucosidase) - 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 (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 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°C results in no detectable activity as determined by thin layer chromatography.

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Glycosidase Activity (α1-2 Fucosidase) - A 10 µl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-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 (α1-3 Fucosidase) - 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°C results in no detectable activity as determined by thin layer chromatography.
Glycosidase Activity (α1-3 Galactosidase) - A 10 µl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-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 (α1-3 Mannosidase) - A 10 µl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-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.
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.
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 (Manα1-6Manα1-6(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.
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.
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.
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.
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.

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

Date 17 Oct 2024

Lauren Brown
Quality Approver