

New England Biolabs Product Specification

Product Name:	<i>O-Glycosidase</i>
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).
Shelf Life:	24 months
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:	23 Dec 2015

Assay Name/Specification (minimum release criteria)

Glycosidase Activity (Endo F1, F2, H) - 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.

Glycosidase Activity (Endo F2, F3) - 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.

Glycosidase Activity (PNGase F) - 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.

Glycosidase Activity (β-Mannosidase) - 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.

Glycosidase Activity (β-N-Acetylgalactosaminidase) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-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.



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Glycosidase Activity (β -N-Acetylglucosaminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -N-Acetylglucosaminidase substrate (GlcNAc β 1-4GlcNAc β 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.

Glycosidase Activity (β -Xylosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -Xylosidase substrate (Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-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.

Glycosidase Activity (β 1-3 Galactosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-3GlcNAc β 1-4Gal β 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.

Glycosidase Activity (β 1-4 Galactosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-4GlcNAc β 1-3Gal β 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.

Glycosidase Activity (α -Glucosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Glucosidase substrate (Glc α 1-6Glc α 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.

Glycosidase Activity (α -N-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -N-Acetylgalactosaminidase substrate (GalNAc α 1-3(Fuc α 1-2)Gal β 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.

Glycosidase Activity (α -Neuraminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Neuraminidase substrate (Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 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.

Glycosidase Activity (α 1-2 Fucosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc α 1-2Gal β 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.

Glycosidase Activity (α 1-3 Fucosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc α 1-3Gal β 1-4GlcNAc β 1-3Gal β 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.

Glycosidase Activity (α 1-3 Galactosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-3Gal β 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.



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Glycosidase Activity (α 1-3 Mannosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-3Man β 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.

Glycosidase Activity (α 1-6 Galactosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-6Gal α 1-6Glc α 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.

Glycosidase Activity (α 1-6 Mannosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-6Man α 1-6(Man α 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.

Protease Activity (SDS-PAGE) - A 20 μ l reaction in 1X Glyco Buffer 2 containing 24 μ 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.

Protein Purity Assay (SDS-PAGE) - O-Glycosidase is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



Date 23 Dec 2015

Derek Robinson
Director of Quality Control

