

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>O-Glycoprotease</i>
<i>Catalog #:</i>	<i>P0761S</i>
<i>Concentration:</i>	<i>1,000 units/ml</i>
<i>Unit Definition:</i>	<i>One unit of O-Glycoprotease will cleave &gt;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.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>20 mM Tris-HCl, 100 mM NaCl (pH 7.5 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-P0761S v1.0</i>
<i>Effective Date:</i>	<i>19 Jan 2021</i>

### 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 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 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 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 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 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**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 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.



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**Glycosidase Activity ( $\alpha$ 1-3 Fucosidase)** - A 10  $\mu$ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled  $\alpha$ -Fucosidase substrate (Fu $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 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.

**Glycosidase Activity ( $\alpha$ 1-3 Galactosidase)** - 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.

**Glycosidase Activity ( $\alpha$ 1-3 Mannosidase)** - 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.

**Glycosidase Activity ( $\alpha$ 1-6 Galactosidase)** - 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.

**Glycosidase Activity ( $\alpha$ 1-6 Mannosidase)** - 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 $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 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.

**Glycosidase Activity ( $\alpha$ -*N*-Acetylgalactosaminidase)** - A 10  $\mu$ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled  $\alpha$ -*N*-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fu $\alpha$ 1-2)Gal $\beta$ 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.

**Glycosidase Activity ( $\beta$ -Mannosidase)** - A 10  $\mu$ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled  $\beta$ -Mannosidase substrate (Man $\beta$ 1-4Man $\beta$ 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.

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

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

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



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**Glycosidase Activity ( $\beta$ -N-Acetylgalactosaminidase)** - A 10  $\mu$ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled  $\beta$ -N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 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.

**Glycosidase Activity ( $\beta$ -N-Acetylglucosaminidase)** - A 10  $\mu$ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled  $\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 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.

**Protease Activity (Non-Specific, SDS-PAGE)** - A 20  $\mu$ l reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 24  $\mu$ 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.

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