New England Biolabs
Product Specification

Product Name: O-Glycoprotease
Catalog #: P0761S
Concentration: 1,000 units/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.
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 v1.0
Effective Date: 19 Jan 2021

<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
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<tbody>
<tr>
<td><strong>Glycosidase Activity (Endo F1, F2, H)</strong> - 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.</td>
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<td><strong>Glycosidase Activity (Endo F2, F3)</strong> - 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.</td>
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<td><strong>Glycosidase Activity (PNGase F)</strong> - 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.</td>
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<td><strong>Glycosidase Activity (α-Glucosidase)</strong> - A 10 µl 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.</td>
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<td><strong>Glycosidase Activity (α-Neuraminidase)</strong> - 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.</td>
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<td><strong>Glycosidase Activity (α1-2 Fucosidase)</strong> - 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.</td>
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### Glycosidase Activity (α-1-3 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 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 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 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 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 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 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 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 µl reaction in 20 mM Tris-HCl (pH 8.0 @ 25°C) containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-6Manα1-6Manα1-3Man-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-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 (GalNAccα1-3(Fucα1-2)Galβ1-4Glc-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 (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 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 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 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 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 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 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 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 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 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 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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Derek Robinson  
**Director, Quality Control**

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Date 19 Jan 2021