O-Glycosidase

Source: Cloned from Enterococcus faecalis and expressed in E. coli (1).

Applications:
- Removal of Core 1 & Core 3 O-linked disaccharide glycans from glycoproteins

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 1 mM Na2EDTA.

Reagents Supplied with Enzyme:
10X Glycoprotein Denaturing Buffer
10X G7 Reaction Buffer
10% NP-40

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 fetuin (2) 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).

Molecular Weight: 147,000 daltons.

Quality Assurance: No contaminating exoglycosidase or proteolytic activity could be detected.

Quality Controls
Glycosidase Assays:
200,000 units of O-Glycosidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

No other glycosidase activities were detected (ND) with the following substrates:
- β-N-Acetylgalactosaminidase:
  - GalNacβ1-4Galβ1-4Glc-AMC
  - ND
- α-N-Acetylgalactosaminidase:
  - GalNAcα1-3(Fucx1-2)Galβ1-4Glc-AMC
  - ND
- α-Fucosidase:
  - Fucx1-2Galβ1-4Glc-AMC
  - ND
  - Galβ1-4 (Fucx1-3)GlcNAcβ1-3Galβ1-4Glc-AMC
  - ND

Non-Denaturing Unit Definition Assay: Two fold serial dilutions of O-Glycosidase are added to a reaction mixture of 5 mg of neuraminidase digested fetuin with 1X G7 Reaction Buffer. The reaction mix is then incubated at 37°C for 1 hour. O-linked disaccharide carbohydrates are determined by the Morgan and Elson Assay (2).

Note: Under denaturing conditions the enzyme activity increased two-fold. This observation is substrate dependent.

Specific Activity: 53,000,000 units/mg.
Molecular Weight: 147,000 daltons.
Quality Assurance: No contaminating exoglycosidase or proteolytic activity could be detected.

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  - ND
- α-Fucosidase:
  - Fucx1-2Galβ1-4Glc-AMC
  - ND
  - Galβ1-4 (Fucx1-3)GlcNAcβ1-3Galβ1-4Glc-AMC
  - ND

(see other side)
**β-Galactosidase:**

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<tr>
<th>Compound</th>
<th>Activity</th>
<th>Notes</th>
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<tr>
<td>Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC</td>
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<tr>
<td>Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC</td>
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**α-Galactosidase:**

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<tr>
<td>Galα1-3Galβ1-4Gal-AMC</td>
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<td>Galα1-6Galα1-6Glcα1-2Fru-AMC</td>
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**β-Mannosidase:**

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<tr>
<td>Manβ1-4Manβ1-4Man-AMC</td>
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**Endo F₁, F₂, H:**

- Dalsyalted invertase high mannose. ND

**Endo F₃, F₄:**

- Dalsyalted fibrinogen biantennary. ND

**PNGase F:**

- Fluoresceinated fetuin triantennary. ND

**Protease Assay:**

- After incubation of 1,400,000 units of O-Glycosidase with 0.2 nmol of a standard mixture of proteins in a 20 µl reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

**Protocol:**

1. Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate. Typical reaction conditions are as follows:

   1. Combine 10–20 µg of glycoprotein, 1 µl of 10X Glycoprotein Denaturing Buffer and H₂O (if necessary) to make a 10 µl total reaction volume.
   2. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
   3. Make a total reaction volume of 20 µl by adding 2 µl 10X G7 Reaction Buffer, 2 µl 10% NP40, 2 µl Neuraminidase, H₂O and 1–5 µl O-Glycosidase.
   4. Incubate reaction at 37°C for 1–4 hours.

**Notes on Use:**

- Since O-Glycosidase is inhibited by SDS, it is essential to have NP-40 in the reaction mixture. It is not known why this non-ionic detergent counteracts the SDS inhibition at the present time. Double digest with Endo H must have NP-40 present (NP-40 does not inhibit Endo H).
- To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.
- Recommended storage temperature is ~20°C.

**Heat Inactivation:**

- 65°C for 10 minutes.

**References:**


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**β-Glucosidase:**

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<th>Compound</th>
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<tr>
<td>Glcβ1-4Glcβ1-4Glc-AMC</td>
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<td>Glcα1-6Glcα1-4Glc-AMC</td>
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**β-Xylosidase:**

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<td>Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC</td>
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<tbody>
<tr>
<td>Manβ1-4Manβ1-4Man-AMC</td>
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