**α1-6 Mannosidase**

**P0727S**

**Description:** α1-6 Mannosidase is a highly specific exoglycosidase that removes unbranched α1-6-linked α-mannopyranosyl residues from oligosaccharides (1,2). When used in conjunction with α1-2,3 Mannosidase, the α1-6 Mannosidase will cleave α1-6 Mannose residues from branched carbohydrate substrates.

**Note:** Concentration and Specificity Changes

**Specificity:**

\[ \text{Man } \alpha 1 \rightarrow 6 \beta R \]

**Detailed Specificity:** Specificity can vary depending on incubation time and concentration of substrate (Figure 1).

**A. 0.1 nm/µl substrate, 4 hour incubation**

- Man(1→6)Man(1→6)Man(1→3)\text{Man}\]
- Man(1→6)Man(1→3)\text{Man}\]
- Man(1→6)Man(1→3)\text{Man}\]
- Man(1→6)Man(1→3)\text{Man}\]

**B. 0.1 nm/µl substrate, 4 hour incubation**

- Man(1→6)Man(1→6)Man(1→3)\text{Man}\]
- Man(1→6)Man(1→3)\text{Man}\]
- Man(1→6)Man(1→3)\text{Man}\]
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**C. 0.1 nm/µl substrate, 18 hour incubation**

- Man(1→6)Man(1→6)Man(1→3)\text{Man}\]
- Man(1→6)Man(1→3)\text{Man}\]
- Man(1→6)Man(1→3)\text{Man}\]
- Man(1→6)Man(1→3)\text{Man}\]

**D. 0.05 nm/µl substrate, 18 hour incubation**

- Man(1→2)Man(1→6)Man(1→3)\text{Man}\]
- Man(1→2)Man(1→3)\text{Man}\]
- Man(1→2)Man(1→3)\text{Man}\]
- Man(1→2)Man(1→3)\text{Man}\]

**E. 0.045 nm/µl substrate, 18 hour incubation**

- Man(1→2)Man(1→3)Man(1→6)\text{Man}\]
- Man(1→2)\text{Man}\]
- Man(1→2)\text{Man}\]
- Man(1→2)\text{Man}\]

**Note:** Concentration and Specificity Changes

**Source:** Cloned from *Xanthomonas manihotis* and expressed in *E. coli* (2).

**Supplied in:** 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C), 0.1 mM Na₂EDTA.

**Reagents Supplied with Enzyme:**

- 10X G2 Reaction Buffer
- 100X BSA

**Reaction Conditions:**

- 1X G2 Reaction Buffer: 50 mM Sodium Citrate (pH 4.5 @ 25°C). Supplement with 100 µg/ml BSA. Incubate at 37°C.

**Note:** A double digest with α1-2,3 Mannosidase requires the following reaction conditions: 1X G6 Reaction Buffer: 50 mM Sodium Acetate (pH 5.5 @ 25°C), 5 mM CaCl₂. Supplement with 100 µg/ml BSA. Incubate at 37°C.

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**D. 0.05 nm/µl substrate, 18 hour incubation**

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- Man(1→2)Man(1→3)Man(1→6)\text{Man}\]
- Man(1→2)\text{Man}\]
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**Note:** p-nitrophenyl-α-D-mannopyranoside is NOT a substrate for this enzyme.

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(see other side)
### Quality Controls

#### Glycosidase Assays:
80 units of α1,6-Mannosidase 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.

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<tr>
<th>Enzyme Name</th>
<th>Reaction Products</th>
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<tr>
<td>α-Mannosidase</td>
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<td>Protease Assay</td>
<td>After incubation of 560 units of α1,6-Mannosidase with 0.2 nmol of a standard mixture of proteins for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.</td>
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#### References:

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**Unit Definition:** One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-D-mannose from 1 nmol of Manα1-6Manα1-6Man7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

**Specific Activity:** ~ 137,000 units/mg

**Molecular Weight:** 51,000 daltons

**Unit Definition Assay:** Two fold dilutions of α1,6-Mannosidase are incubated with 1 nmol AMC-labeled substrate in 1X G2 Reaction Buffer, supplemented with 100 µg/ml BSA, in a 10 µl reaction. The reaction mix is incubated for 1 hour at 37°C. Separation of reaction products are visualized via thin layer chromatography (1).

**Quality Assurance:** No contaminating exoglycosidase or proteolytic activity could be detected (ND).

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