α1-2,3 Mannosidase

**Description:** α1-2,3 Mannosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-2 and α1-3 linked β-mannopyranosyl residues from oligosaccharides (1).

**Specificity:**

- \( \alpha_1 \rightarrow 2 R \) (α1-2 R)
- \( \alpha_1 \rightarrow 3 R \) (α1-3 R)

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

**A. 0.1 nm/µl substrate, 1 hour incubation**

\[
\text{Man}_{1-6} \rightarrow \text{Man}_{1-3} \rightarrow \text{Man}_{1-6} \rightarrow \text{Man}_{1-3} \rightarrow \text{Man}_{1-6} \rightarrow \text{GlcNAc} \rightarrow \text{GlcNAc}
\]

**B. 0.1 nm/µl substrate, 1 hour incubation**

\[
\text{Man}_{1-6} \rightarrow \text{Man}_{1-3} \rightarrow \text{Man}_{1-6} \rightarrow \text{Man}_{1-3} \rightarrow \text{Man}_{1-6} \rightarrow \text{GlcNAc} \rightarrow \text{GlcNAc}
\]

**C. 0.1 nm/µl substrate, 18 hour incubation**

\[
\text{Man}_{1-6} \rightarrow \text{Man}_{1-3} \rightarrow \text{Man}_{1-6} \rightarrow \text{Man}_{1-3} \rightarrow \text{Man}_{1-6} \rightarrow \text{GlcNAc} \rightarrow \text{GlcNAc}
\]

**D. 0.1 nm/µl substrate, 18 hour incubation**

\[
\text{Man}_{1-2} \rightarrow \text{Man}_{1-6} \rightarrow \text{Man}_{1-2} \rightarrow \text{Man}_{1-3} \rightarrow \text{Man}_{1-6} \rightarrow \text{GlcNAc} \rightarrow \text{GlcNAc}
\]

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

\[
\text{Man}_{1-2} \rightarrow \text{Man}_{1-3} \rightarrow \text{Man}_{1-2} \rightarrow \text{Man}_{1-3} \rightarrow \text{Man}_{1-2} \rightarrow \text{Man}_{1-3} \rightarrow \text{Man}_{1-3} \rightarrow \text{GlcNAc} \rightarrow \text{GlcNAc}
\]

**Figure 1:** Detailed specificity of α1,2-3 Mannosidase. All reactions contained 32 units of α1,2-3 Mannosidase, 1X G6 Reaction Buffer and 1X BSA in a total reaction volume of 10 µl. Reactions were incubated at 37°C. The substrate depicted in (E) will not cut to completion.

**Note:** p-nitrophenyl-α-D-mannopyranoside is NOT a substrate for this enzyme.

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

**Reagents Supplied with Enzyme:**

- 1X G6 Reaction Buffer
- 100X BSA

**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.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.
Quality Controls

Glycosidase Assays:
32 units of α1-2,3 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.

No other glycosidase activities were detected (ND) with the following substrates:

β-N-Acetylglucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-N-Acetylglactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC
Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:
Galtβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND
Galtβ1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Galg1-3Gal-AMC ND

β-Mannosidase:
Manβ1-4Manβ1-4Man-AMC ND

Endo F1, F2, H:
Dansylated invertase high mannose. ND

Endo Fv, Fp:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 220 units of α1-2,3 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.

References:

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