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 α-D-mannopyranosyl residues from oligosaccharides (1).

Specificity:

\[
\begin{align*}
\alpha 1\rightarrow2 R \\
\alpha 1\rightarrow3 R
\end{align*}
\]

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

A. 0.1 nm/µl substrate, 1 hour incubation

\[
\begin{align*}
\text{Man}\alpha 1\rightarrow6 &\quad \text{Man}\alpha(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc} \\
\text{Man}\alpha 1\rightarrow3 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc} \\
\text{Man}\beta 1\rightarrow3 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc}
\end{align*}
\]

B. 0.1 nm/µl substrate, 1 hour incubation

\[
\begin{align*}
\text{Man}\alpha 1\rightarrow6 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc} \\
\text{Man}\alpha 1\rightarrow3 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc} \\
\text{Man}\alpha 1\rightarrow3 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc}
\end{align*}
\]

C. 0.1 nm/µl substrate, 18 hour incubation

\[
\begin{align*}
\text{Man}\alpha 1\rightarrow6 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc} \\
\text{Man}\alpha 1\rightarrow3 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc} \\
\text{Man}\alpha 1\rightarrow3 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc}
\end{align*}
\]

D. 0.1 nm/µl substrate, 18 hour incubation

\[
\begin{align*}
\text{Man}\alpha 1\rightarrow6 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc} \\
\text{Man}\alpha 1\rightarrow3 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc} \\
\text{Man}\alpha 1\rightarrow3 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc}
\end{align*}
\]

E. 0.045 nm/µl substrate, 18 hour incubation

\[
\begin{align*}
\text{Man}\alpha 1\rightarrow6 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc} \\
\text{Man}\alpha 1\rightarrow3 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc} \\
\text{Man}\alpha 1\rightarrow3 &\quad \text{Man}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow4)\text{GlcNAc}
\end{align*}
\]

Figure 1: Detailed specificity of 1,2-3 Mannosidase. All reactions contained 32 units of 1,2-3 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).

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

Reagents Supplied with Enzyme:

1X G6 Reaction Buffer
100X BSA

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Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the non-reducing terminal α-D-Mannose from 1 nmol Manca1-3Manb1-4GlcNAc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Specific Activity: ~ 80,000 units/mg

Molecular Weight: 90,000 daltons.

Unit Definition Assay: Two fold serial dilutions of α-1,2,3 Mannosidase are incubated with 1 nmol AMC-labeled substrate in 1X G6 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).

Quality Controls

Glycosidase Assays: 32 units of α-1,2,3 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:

\[ \text{β-N-Acetylglucosaminidase:} \]
GlcNAcb1-4GlcNAcb1-4GlcNA-AMC ND

\[ \text{-N-Acetylgalactosaminidase:} \]
GalNAcb1-3(Fucx1-2)Galb1-4Glc-AMC ND

\[ \text{α-Fucosidase:} \]
Fucx1-2Galb1-4Glc-AMC
Galb1-4(Fucx1-3)GlcNAcb1-3Galb1-4Glc-AMC ND

\[ \text{β-Galactosidase:} \]
Galb1-3GlcNAcb1-4Galb1-4Glc-AMC ND

\[ \text{α-Mannosidase:} \]
Manb1-6Manb1-6(Manc1-3)Manb1-4GlcNAc-AMC ND

\[ \text{-Mannosidase:} \]
Manb1-4Manb1-4Man-AMC ND

Endo F3, F4, H:
Dansylated invertase high mannose. ND

Endo F3, F4:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 220 units of α-1,2,3 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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