**α1-2,3 Mannosidase**

640 units 32,000 U/ml Lot: 0161206

RECOMBINANT Store at 4°C Exp: 6/13

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

Specificity:

\[
\text{Man} \quad \alpha 1-2 R \\
\alpha 1-3 R
\]

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

A. 0.1 nm/µl substrate, 1 hour incubation

\[
\begin{align*}
\text{Man}1\alpha6 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc} \\
\text{Man}1\beta3 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc}
\end{align*}
\]

B. 0.1 nm/µl substrate, 1 hour incubation

\[
\begin{align*}
\text{Man}1\alpha6 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc} \\
\text{Man}1\beta3 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc}
\end{align*}
\]

C. 0.1 nm/µl substrate, 18 hour incubation

\[
\begin{align*}
\text{Man}1\alpha6 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc} \\
\text{Man}1\beta3 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc}
\end{align*}
\]

D. 0.1 nm/µl substrate, 18 hour incubation

\[
\begin{align*}
\text{Man}1\alpha6 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc} \\
\text{Man}1\beta3 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc}
\end{align*}
\]

E. 0.045 nm/µl substrate, 18 hour incubation

\[
\begin{align*}
\text{Man}1\alpha6 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc} \\
\text{Man}1\beta3 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc}
\end{align*}
\]

**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

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.

---

**α1-2,3 Mannosidase**

640 units 32,000 U/ml Lot: 0161206

RECOMBINANT Store at 4°C Exp: 6/13

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

Specificity:

\[
\text{Man} \quad \alpha 1-2 R \\
\alpha 1-3 R
\]

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

A. 0.1 nm/µl substrate, 1 hour incubation

\[
\begin{align*}
\text{Man}1\alpha6 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc} \\
\text{Man}1\beta3 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc}
\end{align*}
\]

B. 0.1 nm/µl substrate, 1 hour incubation

\[
\begin{align*}
\text{Man}1\alpha6 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc} \\
\text{Man}1\beta3 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc}
\end{align*}
\]

C. 0.1 nm/µl substrate, 18 hour incubation

\[
\begin{align*}
\text{Man}1\alpha6 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc} \\
\text{Man}1\beta3 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc}
\end{align*}
\]

D. 0.1 nm/µl substrate, 18 hour incubation

\[
\begin{align*}
\text{Man}1\alpha6 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc} \\
\text{Man}1\beta3 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc}
\end{align*}
\]

E. 0.045 nm/µl substrate, 18 hour incubation

\[
\begin{align*}
\text{Man}1\alpha6 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc} \\
\text{Man}1\beta3 & \quad \text{Man}1\beta(1-4)\text{GlcNAc} & \quad \text{Man}1\beta(1-4)\text{GlcNAc}
\end{align*}
\]

**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.

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**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

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.

---

**CERTIFICATE OF ANALYSIS**

1-800-632-7799 info@neb.com www.neb.com

[p-nitrophenyl-α-D-mannopyranoside is NOT a substrate for this enzyme.]

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

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.

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**CERTIFICATE OF ANALYSIS**

1-800-632-7799 info@neb.com www.neb.com
**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 Manca1-3Manβ1-4GlcNAc-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.

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

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**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-Galactosidase:** Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND
- **β-Galactosidase:** Galβ1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAc-AMC ND
- **α-Galactosidase:** Galα1-3Galβ1-4Glc-AMC ND
- **α-Mannosidase:** Manα1-6Manα1-6(Manα1-3)Man-AMC ND
- **α-Neuraminidase:** Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC ND
- **β-Glucosidase:** Glcβ1-4Glcβ1-4Glc-AMC ND
- **β-Glucosidase:** Glcα1-6Galβ1-4Glc-AMC ND
- **β-Xylosidase:** Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND
- **β-Mannosidase:** Manβ1-4Manβ1-4Man-AMC ND
- **Endo F1, F2, H:** Dansylated invertase high mannose. ND
- **Endo F1, F2:** 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:**

U.S. Patent No. 7,094,563

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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 Manca1-3Manβ1-4GlcNAc-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 α1-2,3 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).

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**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(Fucxα1-2)Galβ1-4Glc-AMC ND
- **α-Fucosidase:** Fucxα1-2Galβ1-4Glc-AMC ND
- **α-Fucosidase:** Galβ1-4(Fucxα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND
- **β-Fucosidase:** Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND
- **β-Mannosidase:** Manβ1-4Manβ1-4Man-AMC ND
- **Endo F1, F2, H:** Dansylated invertase high mannose. ND
- **Endo F1, F2:** 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:**

U.S. Patent No. 7,094,563