α1-2,3 Mannosidase

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P0729S 017131014101

P0729S

640 units	**32,000 U/ml**	**Lot: 0171310**
RECOMBINANT	**Store at 4°C**	**Exp: 10/14**

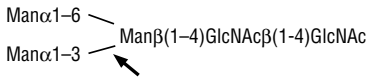
Description: α1-2,3 Mannosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-2 and α1-3 linked D-mannopyranosyl residues from oligosaccharides (1).

Specificity:

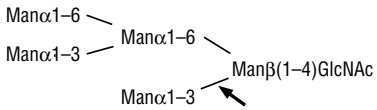
Man α 1– 2 R
α 1– 3 R

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

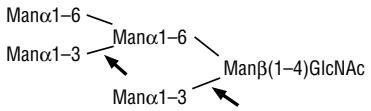
A. 0.1 nm/µl substrate, 1 hour incubation



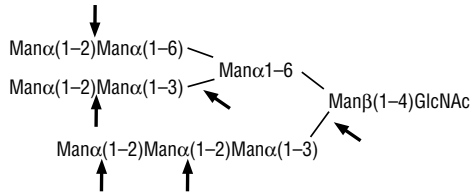
B. 0.1 nm/µl substrate, 1 hour incubation



C. 0.1 nm/µl substrate, 18 hour incubation



D. 0.1 nm/µl substrate, 18 hour incubation



E. 0.045 nm/µl substrate, 18 hour incubation

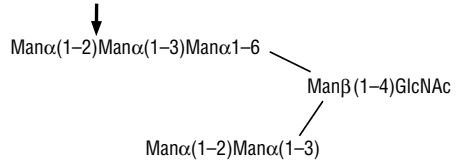


Figure 1: *Detailed specificity of α1,2-3 Mannosidase. All reactions contained 32 units of α1,2-3 Mannosidase, 1X G6 Reaction Buffer and 1X BSA in a total reaction volume of 10 µl. Reactions were incubated at 37°C. The substrate depicted in (E) will not cut to completion.*

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

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

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 1 mM Na_2EDTA.

Reagents Supplied with Enzyme:
10X G6 Reaction Buffer
100X BSA

Reaction Conditions:
1X G6 Reaction Buffer
50 mM Sodium Acetate (pH 5.5 @ 25°C),
5 mM $CaCl_2$. Supplement with 100 µg/ml BSA.
Incubate at 37°C.

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

(see other side)

CERTIFICATE OF ANALYSIS

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Man α 1– 2 R
α 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
B. 0.1 nm/µl substrate, 1 hour incubation
C. 0.1 nm/µl substrate, 18 hour incubation
D. 0.1 nm/µl substrate, 18 hour incubation
E. 0.045 nm/µl substrate, 18 hour incubation
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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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.
(see other side)
CERTIFICATE OF ANALYSIS

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 Manα1-3Manβ1-4GlcNAc-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).

Quality Controls

Glycosidase Assays:
32 units of α1-2,3 Mannosidase were incubated with 0.1 mM of flourescently-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-Acetylgalactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC ND

Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

Galβ1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Galα1-3Gal-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

α-Glucosidase:
Glcα1-6Glcα1-4Glc-AMC ND

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

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

Endo F_1, F_2, H:
Dansylated invertase high mannose. ND

Endo F_2, F_3:
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:

1. Wong-Madden, S.T. and Landry, D. (1995) *Glycobiology* 5, 19–28.
2. Guthrie, E.P., Taron, C.H., New England Biolabs, Inc. unpublished results.

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

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 Manα1-3Manβ1-4GlcNAc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

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Quality Controls

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

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC ND

Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

Galβ1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Galα1-3Gal-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

α-Glucosidase:
Glcα1-6Glcα1-4Glc-AMC ND

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

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

Endo F_1, F_2, H:
Dansylated invertase high mannose. ND

Endo F_2, F_3:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

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