

α1-2,3 Mannosidase



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P0729S 016130514051

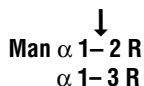
P0729S



640 units **32,000 U/ml** **Lot: 0161305**
RECOMBINANT **Store at 4°C** **Exp: 5/14**

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:



α1-2,3 Mannosidase



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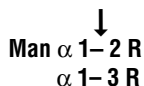
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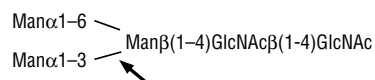
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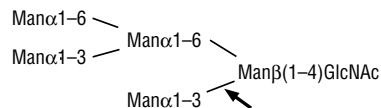


Detailed Specificity: Specificity can vary depending on incubation time and concentration of substrate (Figure 1).

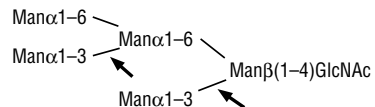
A. 0.1 nm/μl substrate, 1 hour incubation



B. 0.1 nm/μl substrate, 1 hour incubation

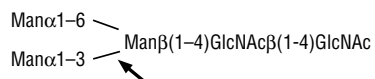


C. 0.1 nm/μl substrate, 18 hour incubation

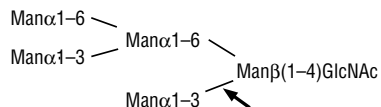


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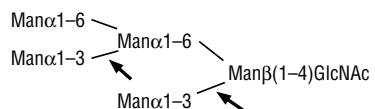
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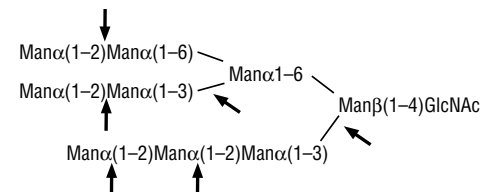
B. 0.1 nm/μl substrate, 1 hour incubation



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

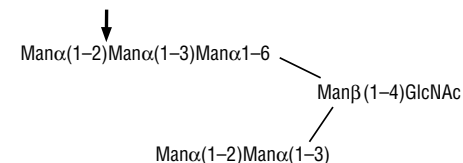
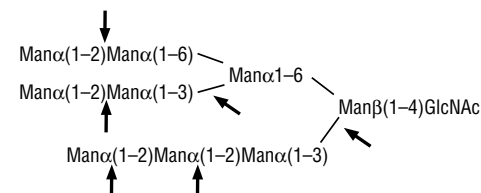


Figure 1: Detailed specificity of α1,2-3 Mannosidase. All reactions contained 32 units of α1,2-3 Mannosidase, 1X G6 Reaction Buffer and 1X BSA in a total reaction volume of 10 μl. Reactions were incubated at 37°C. The substrate depicted in (E) will not cut to completion.

D. 0.1 nm/μl substrate, 18 hour incubation



E. 0.045 nm/μl substrate, 18 hour incubation

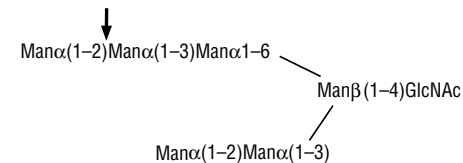


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Note: p-nitrophenyl-α-D-mannopyranoside is NOT a substrate for this enzyme.

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:

10X 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.

(see other side)

CERTIFICATE OF ANALYSIS

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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 Man α 1-3Man β 1-4GlcNAc-7-amino-4-methylcoumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 μ l.

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 μ g/ml BSA, in a 10 μ l 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 α 1-2,3 Mannosidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 μ l 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:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

α -N-Acetylgalactosaminidase:
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC ND

Gal β 1-4 (Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

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GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

α -N-Acetylgalactosaminidase:
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC ND

Gal β 1-4 (Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND

Gal β 1-4GlcNAc β 1-2Man α 1-6Man β 1-4GlcNAc-AMC ND

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal α 1-3Gal-AMC ND

α -Mannosidase:
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

α -Neuraminidase:
Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Glucosidase:
Glc β 1-4Glc β 1-4Glc-AMC ND

α -Glucosidase:
Glc α 1-6Glc α 1-4Glc-AMC ND

β -Xylosidase:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND

Gal β 1-4GlcNAc β 1-2Man α 1-6Man β 1-4GlcNAc-AMC ND

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal α 1-3Gal-AMC ND

α -Mannosidase:
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

α -Neuraminidase:
Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Glucosidase:
Glc β 1-4Glc β 1-4Glc-AMC ND

α -Glucosidase:
Glc α 1-6Glc α 1-4Glc-AMC ND

β -Xylosidase:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

β -Mannosidase:
Man β 1-4Man β 1-4Man-AMC ND

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

Endo F₂, F₃:
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:

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

β -Mannosidase:
Man β 1-4Man β 1-4Man-AMC ND

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

Endo F₂, F₃:
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.

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