α1-6 Mannosidase

1-800-632-7799
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P0727S 009121013101

P0727S

800 units	**40,000 U/ml**	**Lot: 0091210**
RECOMBINANT	**Store at 4°C**	**Exp: 10/13**

Description: α1-6 Mannosidase is a highly specific exoglycosidase that removes unbranched α1-6 linked D-mannopyranosyl residues from oligosaccharides (1,2). When used in conjunction with α1-2,3 Mannosidase, the α1-6 Mannosidase will cleave α1-6 Mannose residues from branched carbohydrate substrates.

Note: Concentration and Specificity Changes

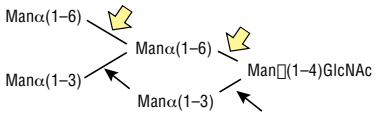
Specificity:

Man α 1 – 6 R

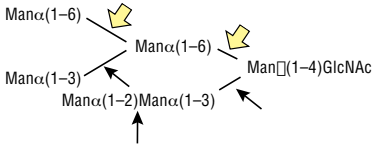
Detailed Specificity: Specificity can vary depending on incubation time and concentration of substrate (Figure 1).

A. 0.1 nm/µl substrate, 4 hour incubation

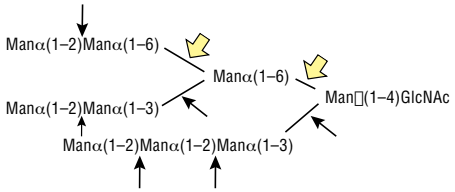
B. 0.1 nm/µl substrate, 4 hour incubation



C. 0.1 nm/µl substrate, 18 hour incubation



D. 0.05 nm/µl substrate, 18 hour incubation



E. 0.045 nm/µl substrate, 18 hour incubation

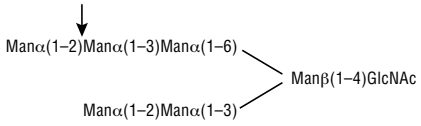


Figure 1: Detailed specificity of α1-6 Mannosidase. *All reactions contained 32 units of α1-2,3 Mannosidase (NEB #P0729), 40 units of α1-6 Mannosidase, 1X G6 reaction buffer and 1X BSA In a total reaction volume of 10 µl. Reactions were incubated at 37°C. The substrate depicted in (E) will not cut to completion. If this structure exists in any substrate it will be impervious to cleavage by α1-6 Mannosidase. Note: When used alone, α1-6 Mannosidase will still act only on linear substrates. When used in conjunction with α1-2,3 Mannosidase, the α1-6 Mannosidase will cleave α1-6 Mannose residues from branched carbohydrate substrates.*

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), 0.1 mM Na_2EDTA.

Reagents Supplied with Enzyme:
10X G2 Reaction Buffer
100X BSA

Reaction Conditions:
1X G2 Reaction Buffer:
50 mM Sodium Citrate (pH 4.5 @ 25°C).
Supplement with 100 µg/ml BSA. Incubate at 37°C.

Note: A double digest with α1-2,3 Mannosidase requires the following 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.

(see other side)

CERTIFICATE OF ANALYSIS

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B. 0.1 nm/µl substrate, 4 hour incubation

C. 0.1 nm/µl substrate, 18 hour incubation

D. 0.05 nm/µl substrate, 18 hour incubation

E. 0.045 nm/µl substrate, 18 hour incubation

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(see other side)

CERTIFICATE OF ANALYSIS

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-D-mannose from 1 nmol of Manα1-6Manα1-6Man-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Specific Activity: ~ 137,000 units/mg

Molecular Weight: 51,000 daltons

Unit Definition Assay: Two fold dilutions of α1-6 Mannosidase are incubated with 1 nmol AMC-labeled substrate in 1X G2 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: 80 units of α1--6 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.

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

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

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

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

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

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3
Galβ1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-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 560 units of α1-6 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

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Molecular Weight: 51,000 daltons

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

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

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

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3
Galβ1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-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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