

α 1-6 Mannosidase



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



P0727S 010140215021

P0727S



800 units **40,000 U/ml** **Lot: 0101402**
RECOMBINANT **Store at 4°C** **Exp: 2/15**

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

Note: Concentration and Specificity Changes

α 1-6 Mannosidase



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



P0727S 010140215021

P0727S



800 units **40,000 U/ml** **Lot: 0101402**
RECOMBINANT **Store at 4°C** **Exp: 2/15**

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

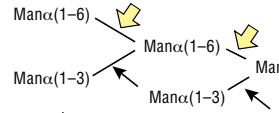
Note: Concentration and Specificity Changes

Specificity:

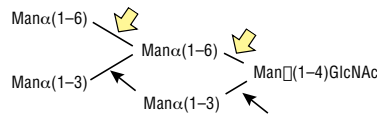
Man α 1 - 6 R

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

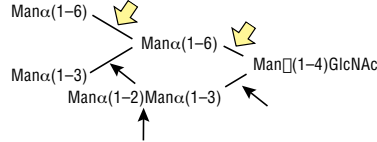
A. 0.1 nm/μl substrate, 4 hour incubation



B. 0.1 nm/μl substrate, 4 hour incubation



C. 0.1 nm/μl substrate, 18 hour incubation

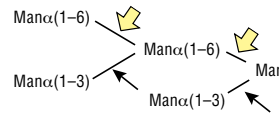


Specificity:

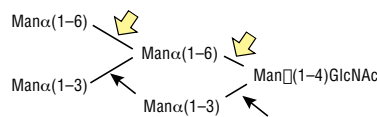
Man α 1 - 6 R

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

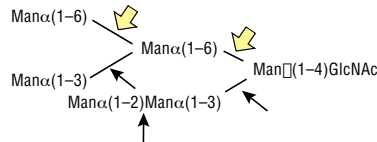
A. 0.1 nm/μl substrate, 4 hour incubation



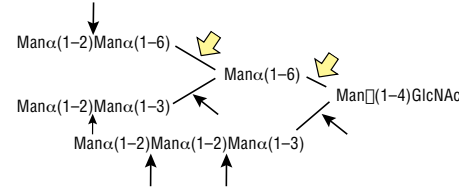
B. 0.1 nm/μl substrate, 4 hour incubation



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

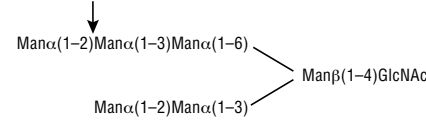
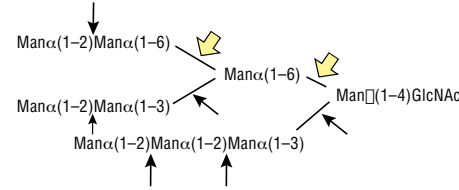


Figure 1: Detailed specificity of α 1-6 Mannosidase. All reactions contained 32 units of α 1-2,3 Mannosidase (NEB #P0729), 40 units of α 1-6 Mannosidase, 1X G6 reaction buffer and 1X BSA in a total reaction volume of 10 μ l. Reactions were incubated at 37°C. The substrate depicted in (E) will not cut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

D. 0.05 nm/μl substrate, 18 hour incubation



E. 0.045 nm/μl substrate, 18 hour incubation

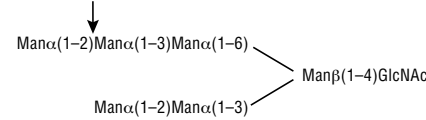


Figure 1: Detailed specificity of α 1-6 Mannosidase. All reactions contained 32 units of α 1-2,3 Mannosidase (NEB #P0729), 40 units of α 1-6 Mannosidase, 1X G6 reaction buffer and 1X BSA in a total reaction volume of 10 μ l. Reactions were incubated at 37°C. The substrate depicted in (E) will not cut to completion. If this structure exists in any substrate it will be impervious to cleavage by α 1-6 Mannosidase. Note: When used alone, α 1-6 Mannosidase will still act only on linear substrates. When used in conjunction with α 1-2,3 Mannosidase, the α 1-6 Mannosidase will cleave α 1-6 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 Na₂EDTA.

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 μ g/ml BSA. Incubate at 37°C.

Note: A double digest with α 1-2,3 Mannosidase requires the following reaction conditions: 1X G6 Reaction Buffer: 50 mM Sodium Acetate (pH 5.5 @ 25°C), 5 mM CaCl₂. Supplement with 100 μ g/ml BSA. Incubate at 37°C.

(see other side)

CERTIFICATE OF ANALYSIS

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 Na₂EDTA.

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 μ g/ml BSA. Incubate at 37°C.

Note: A double digest with α 1-2,3 Mannosidase requires the following reaction conditions: 1X G6 Reaction Buffer: 50 mM Sodium Acetate (pH 5.5 @ 25°C), 5 mM CaCl₂. Supplement with 100 μ g/ml BSA. Incubate at 37°C.

(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 Man α 1-6Man α 1-6Man-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 μ l.

Specific Activity: ~ 137,000 units/mg

Molecular Weight: 51,000 daltons

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

Page 2 (P0727)

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 Man α 1-6Man α 1-6Man-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 μ l.

Specific Activity: ~ 137,000 units/mg

Molecular Weight: 51,000 daltons

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

Page 2 (P0727)

Quality Controls

Glycosidase Assays: 80 units of α 1-6 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.

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:
Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND
Fuc α 1-2Gal β 1-4Glc-AMC ND

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

Quality Controls

Glycosidase Assays: 80 units of α 1-6 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.

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:
Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND
Fuc α 1-2Gal β 1-4Glc-AMC ND

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

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

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

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-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 1-3Gal β 1-4Glc-AMC ND

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

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-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 560 units of α 1-6 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

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 560 units of α 1-6 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