

# $\alpha$ 1-6 Mannosidase



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P0727S 009140715071

## P0727S



**800 units**    **40,000 U/ml**    **Lot: 0091407**  
**RECOMBINANT**    **Store at 4°C**    **Exp: 7/15**

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

**Note: Concentration and Specificity Changes**

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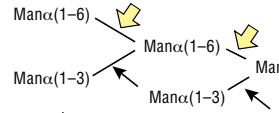
**Note: Concentration and Specificity Changes**

### Specificity:

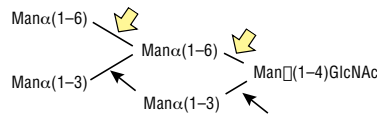
Man  $\alpha$  1 - 6 R

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

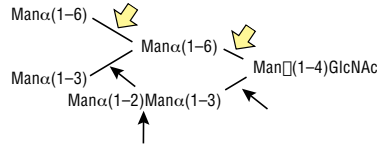
#### A. 0.1 nm/ $\mu$ l substrate, 4 hour incubation



#### B. 0.1 nm/ $\mu$ l substrate, 4 hour incubation



#### C. 0.1 nm/ $\mu$ l substrate, 18 hour incubation

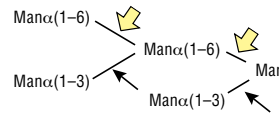


### Specificity:

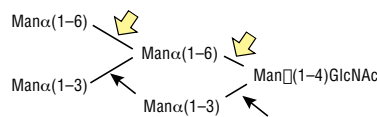
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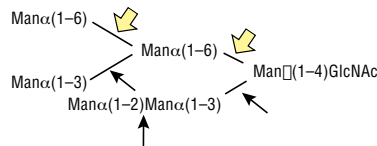
#### A. 0.1 nm/ $\mu$ l substrate, 4 hour incubation



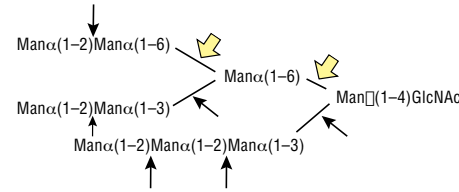
#### B. 0.1 nm/ $\mu$ l substrate, 4 hour incubation



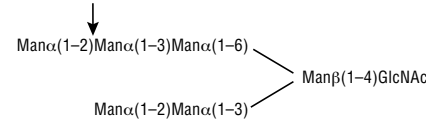
#### C. 0.1 nm/ $\mu$ l substrate, 18 hour incubation



#### D. 0.05 nm/ $\mu$ l substrate, 18 hour incubation

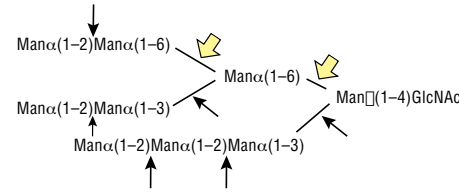


#### E. 0.045 nm/ $\mu$ l substrate, 18 hour incubation

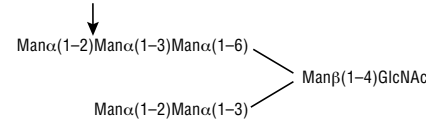


**Figure 1: Detailed specificity of  $\alpha$ 1-6 Mannosidase.** All reactions contained 32 units of  $\alpha$ 1-2,3 Mannosidase (NEB #P0729), 40 units of  $\alpha$ 1-6 Mannosidase, 1X G6 reaction buffer and 1X BSA in a total reaction volume of 10  $\mu$ l. Reactions were incubated at 37°C. The substrate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

#### D. 0.05 nm/ $\mu$ l substrate, 18 hour incubation



#### E. 0.045 nm/ $\mu$ l substrate, 18 hour incubation



**Figure 1: Detailed specificity of  $\alpha$ 1-6 Mannosidase.** All reactions contained 32 units of  $\alpha$ 1-2,3 Mannosidase (NEB #P0729), 40 units of  $\alpha$ 1-6 Mannosidase, 1X G6 reaction buffer and 1X BSA in a total reaction volume of 10  $\mu$ 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  $\alpha$ 1-6 Mannosidase. Note: When used alone,  $\alpha$ 1-6 Mannosidase will still act only on linear substrates. When used in conjunction with  $\alpha$ 1-2,3 Mannosidase, the  $\alpha$ 1-6 Mannosidase will cleave  $\alpha$ 1-6 Mannose residues from branched carbohydrate substrates.

Note: p-nitrophenyl- $\alpha$ -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<sub>2</sub>EDTA.

#### 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  $\mu$ g/ml BSA. Incubate at 37°C.

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

(see other side)

CERTIFICATE OF ANALYSIS

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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  $\alpha$ -D-mannose from 1 nmol of Man $\alpha$ 1-6Man $\alpha$ 1-6Man-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.

**Specific Activity:** ~ 137,000 units/mg

**Molecular Weight:** 51,000 daltons

**Unit Definition Assay:** Two fold dilutions of  $\alpha$ 1-6 Mannosidase are incubated with 1 nmol AMC-labeled substrate in 1X G2 Reaction Buffer, supplemented with 100  $\mu$ g/ml BSA, in a 10  $\mu$ 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:** 80 units of  $\alpha$ 1-6 Mannosidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10  $\mu$ l reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

**$\beta$ -N-Acetylglucosaminidase:**  
GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC ND

**$\alpha$ -N-Acetylgalactosaminidase:**  
GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Fucosidase:**  
Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND  
Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC ND

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**$\beta$ -N-Acetylglucosaminidase:**  
GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC ND

**$\alpha$ -N-Acetylgalactosaminidase:**  
GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Fucosidase:**  
Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND  
Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC ND

**$\beta$ -Galactosidase:**  
Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND  
Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Neuraminidase:**  
Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC ND

**$\beta$ -Glucosidase:**  
Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Glucosidase:**  
Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC ND

**$\beta$ -Xylosidase:**  
Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC ND

**$\beta$ -Galactosidase:**  
Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND  
Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Neuraminidase:**  
Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC ND

**$\beta$ -Glucosidase:**  
Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Glucosidase:**  
Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC ND

**$\beta$ -Xylosidase:**  
Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC ND

**$\beta$ -Mannosidase:**  
Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC ND

**Endo F<sub>1</sub>, F<sub>2</sub>, H:**  
Dansylated invertase high mannose. ND

**Endo F<sub>2</sub>, F<sub>3</sub>:**  
Dansylated fibrinogen biantennary. ND

**PNGase F:**  
Fluoresceinated fetuin triantennary. ND

**Protease Assay:** After incubation of 560 units of  $\alpha$ 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

**$\beta$ -Mannosidase:**  
Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC ND

**Endo F<sub>1</sub>, F<sub>2</sub>, H:**  
Dansylated invertase high mannose. ND

**Endo F<sub>2</sub>, F<sub>3</sub>:**  
Dansylated fibrinogen biantennary. ND

**PNGase F:**  
Fluoresceinated fetuin triantennary. ND

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