

# $\alpha$ 1-2,3 Mannosidase



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P0729S 017150816081

## P0729S



**640 units**    **32,000 U/ml**    **Lot: 0171508**  
**RECOMBINANT**    **Store at 4°C**    **Exp: 8/16**

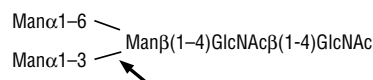
**Description:**  $\alpha$ 1-2,3 Mannosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of  $\alpha$ 1-2 and  $\alpha$ 1-3 linked D-mannopyranosyl residues from oligosaccharides (1).

**Specificity:**  
↓  
**Man  $\alpha$  1-2 R**  
 **$\alpha$  1-3 R**

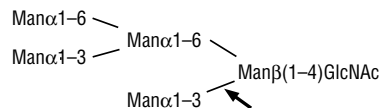
**Note:** p-nitrophenyl- $\alpha$ -D-mannopyranoside is NOT a substrate for this enzyme.

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

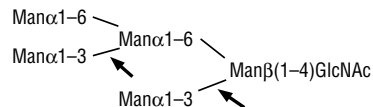
### A. 0.1 nm/ $\mu$ l substrate, 1 hour incubation



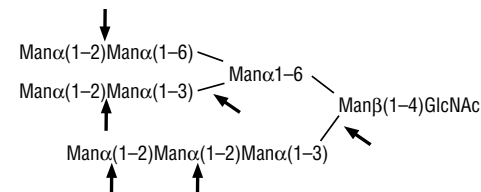
### B. 0.1 nm/ $\mu$ l substrate, 1 hour incubation



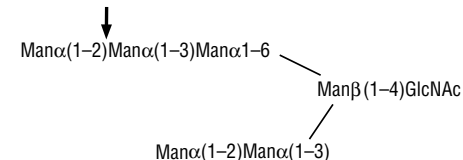
### C. 0.1 nm/ $\mu$ l substrate, 18 hour incubation



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



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



**Figure 1:** Detailed specificity of  $\alpha$ 1,2-3 Mannosidase. All reactions contained 32 units of  $\alpha$ 1,2-3 Mannosidase, 1X GlycoBuffer 1 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.

**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  $\text{Na}_2\text{EDTA}$ .

**Reagents Supplied with Enzyme:**  
10X GlycoBuffer 1  
100X BSA

### Reaction Conditions:

1X GlycoBuffer 1:  
50 mM Sodium Acetate (pH 5.5 @ 25°C)  
and 5 mM  $\text{CaCl}_2$ . Supplement with 100  $\mu\text{g/ml}$  BSA. Incubate at 37°C.

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

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave > 95% of the non-reducing terminal  $\alpha$ -D-Mannose from 1 nmol  $\text{Man}\alpha(1-3)\text{Man}\beta(1-4)\text{GlcNAc}-7\text{-amino-4-methylcoumarin}$  (AMC), in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.

(see other side)  
CERTIFICATE OF ANALYSIS

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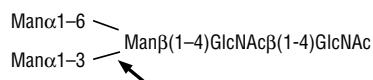
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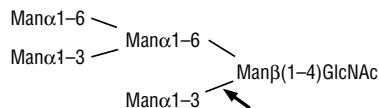
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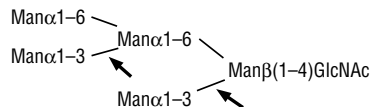
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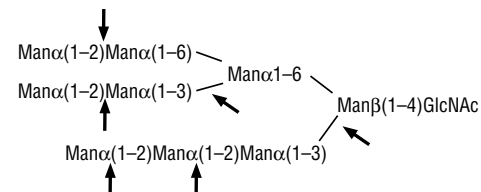
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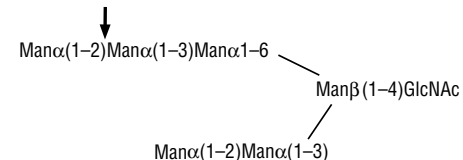
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(see other side)  
CERTIFICATE OF ANALYSIS

**Specific Activity:** ~ 80,000 units/mg

**Molecular Weight:** 90,000 daltons.

**Unit Definition Assay:** Two fold serial dilutions of  $\alpha$ 1-2,3 Mannosidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1, 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).

### Quality Controls

#### Glycosidase Assays:

32 units of  $\alpha$ 1-2,3 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.

Page 2 (P0727)

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Page 2 (P0727)

No other glycosidase activities were detected (ND) with the following substrates:

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

**$\beta$ -Galactosidase:**  
Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND  
Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6Man $\beta$ 1-4GlcNAc-AMC ND

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

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

**$\alpha$ -Neuraminidase:**

No other glycosidase activities were detected (ND) with the following substrates:

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

**$\beta$ -Galactosidase:**  
Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND  
Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6Man $\beta$ 1-4GlcNAc-AMC ND

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

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

**$\alpha$ -Neuraminidase:**

Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-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

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

Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-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

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

**Protease Assay:** After incubation of 220 units of  $\alpha$ 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

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