

# $\alpha$ 1-2 Fucosidase



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P0724S 010150116011

## P0724S

1,000 units    20,000 U/ml    Lot: 0101501  
Store at 4°C    Exp: 1/16

**Description:**  $\alpha$ 1-2 Fucosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of linear  $\alpha$ 1-2 linked L-fucopyranosyl residues from oligosaccharides (1). In this case, a linear substrate is defined as having no branching on the adjacent residue.

New Specificity

# $\alpha$ 1-2 Fucosidase



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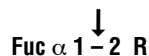
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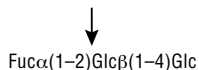
### Specificity:



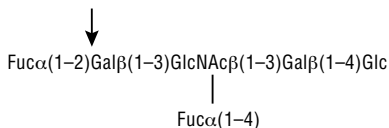
Note: p-nitrophenyl- $\alpha$ -L-fucopyranoside is NOT a substrate for this enzyme.

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

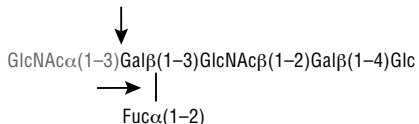
#### A. 0.1 nm/ $\mu$ l substrate, 1 hour incubation



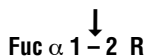
#### B. 0.1 nm/ $\mu$ l substrate, 1 hour incubation



#### C. 0.05 nm/ $\mu$ l substrate, 1 hour incubation



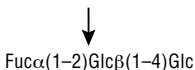
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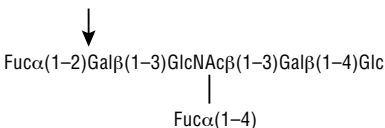
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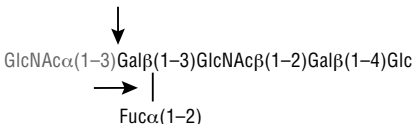
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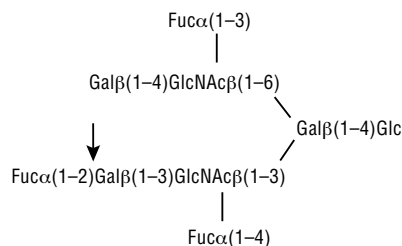
#### B. 0.1 nm/ $\mu$ l substrate, 1 hour incubation



#### C. 0.05 nm/ $\mu$ l substrate, 1 hour incubation



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



**Figure 1: Detailed specificity of  $\alpha$ 1-2 Fucosidase.** Reactions (A), (B) and (D) contained 1X G4 reaction buffer and reactions (C) contained 1X G6 reaction buffer. All reactions contain 1X BSA in a total reaction volume of 10  $\mu$ l, and all reactions were incubated at 37°C. All reactions contained 20 units of  $\alpha$ 1-2 Fucosidase. Reaction (C) also contained 20 units of  $\alpha$ -N-Acetylgalactosaminidase (#P0734). In reaction (C), the branched  $\alpha$ 1-2 fucose is removed in the presence of both enzymes, but not by  $\alpha$ 1-2 Fucosidase alone.

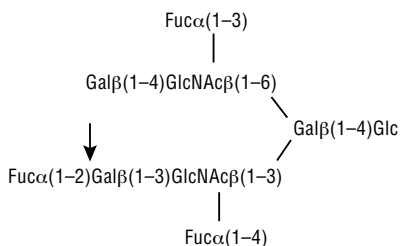
**Source:** Cloned from *Xanthomonas manihotis* and expressed in *E.coli*.

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 0.1 mM Na<sub>2</sub>EDTA.

#### Reagents Supplied with Enzyme:

10X G4 Reaction Buffer  
100X BSA

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



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**Reaction Conditions:** 1X G4 Reaction Buffer: 50 mM Sodium Citrate (pH 6.0 @ 25°C), 100 mM NaCl. Supplement with 100  $\mu$ 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  $\alpha$ -L-fucose from 1 nmol of Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.

**Specific Activity:** ~ 2,000,000 units/mg

**Molecular Weight:** 70,000 daltons

**Unit Definition Assay:** Two fold dilutions of  $\alpha$ 1-2 Fucosidase are incubated with 1 nmol AMC-labeled substrate in 1X G4 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).

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

**Quality Assurance:** No contaminating exoglycosidase or proteolytic activity could be detected.

### Quality Controls

**Glycosidase Assays:** 100 units of  $\alpha$ 1-2 Fucosidase 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.

**Physical Purity:** Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

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

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

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

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

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

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

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

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

**$\alpha$ -Galactosidase:**  
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**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

**$\beta$ -Glucosidase:**  
Glc $\beta$ 1-4Glc $\beta$ 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 100 units of  $\alpha$ 1-2 Fucosidase with 0.2 nmol of a standard mixture of proteins in a 20  $\mu$ l reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

**Note:** Repeated freeze/thaw cycles may reduce activity. Recommended storage temperature has changed to 4°C.

### Reference:

1. Wong-Madden, S.T. and Landry, D. (1995) *Glycobiology* 5, 19–28.

U.S. Patent No. 6,300,113

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