

α 1-2 Fucosidase



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P0724S

1,000 units 20,000 U/ml Lot: 0101310
Store at 4°C Exp: 10/14

Description: α 1-2 Fucosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of linear α 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

α 1-2 Fucosidase



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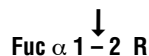
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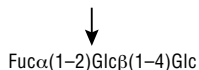
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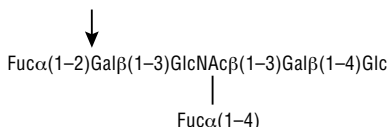
Note: p-nitrophenyl- α -L-fucopyranoside is NOT a substrate for this enzyme.

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

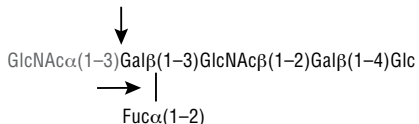
A. 0.1 nm/ μ l substrate, 1 hour incubation



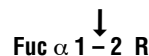
B. 0.1 nm/ μ l substrate, 1 hour incubation



C. 0.05 nm/ μ l substrate, 1 hour incubation



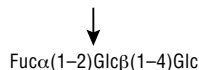
Specificity:



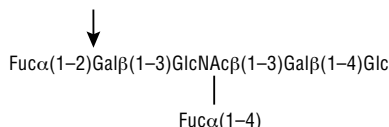
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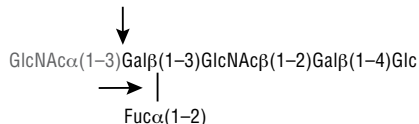
A. 0.1 nm/ μ l substrate, 1 hour incubation



B. 0.1 nm/ μ l substrate, 1 hour incubation



C. 0.05 nm/ μ l substrate, 1 hour incubation



D. 0.1 nm/ μ l substrate, 1 hour incubation

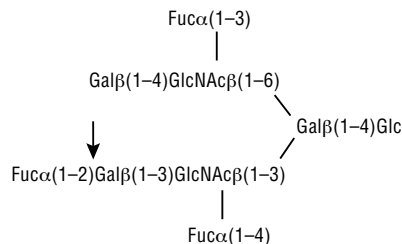


Figure 1: Detailed specificity of α 1-2 Fucosidase. Reactions (A), (B) and (D) contained 1X G4 reaction buffer and reactions (C) contained 1X G6 reaction buffer. All reactions contain 1X BSA in a total reaction volume of 10 μ l, and all reactions were incubated at 37°C. All reactions contained 20 units of α 1-2 Fucosidase. Reaction (C) also contained 20 units of α -N-Acetylgalactosaminidase (#P0734). In reaction (C), the branched α 1-2 fucose is removed in the presence of both enzymes, but not by α 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 Na₂EDTA.

Reagents Supplied with Enzyme:

10X G4 Reaction Buffer
100X BSA

D. 0.1 nm/ μ l substrate, 1 hour incubation

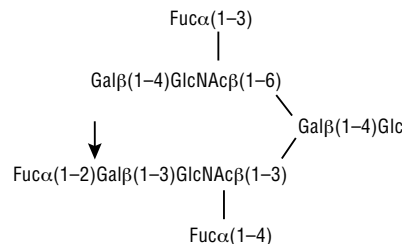


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Reaction Conditions: 1X G4 Reaction Buffer: 50 mM Sodium Citrate (pH 6.0 @ 25°C), 100 mM NaCl. Supplement with 100 μ g/ml BSA. Incubate at 37°C.

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 α -L-fucose from 1 nmol of Fuc α 1-2Gal β 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 μ l.

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

Molecular Weight: 70,000 daltons

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

(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 α 1-2 Fucosidase 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.

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:

β -N-Acetyl-glucosaminidase:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

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

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

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

α -Galactosidase:
Gal α 1-3Gal β 1-4GlcNAc-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
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

β -Glucosidase:
Glc β 1-4Glc β 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

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

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Gal α 1-3Gal β 1-4GlcNAc-AMC ND

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α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

β -Glucosidase:
Glc β 1-4Glc β 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 100 units of α 1-2 Fucosidase with 0.2 nmol of a standard mixture of proteins in a 20 μ 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

Endo F₂, F₃:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

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