α1-2 Fucosidase

NEW ENGLAND BioLabs Inc.
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P0724S 010121013101

P0724S

1,000 units **20,000 U/ml** **Lot: 0101210**
Store at 4°C **Exp: 10/13**

Description: α1-2 Fucosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of linear α1-2 linked L-fucopyranosyl residues from oligosaccharides (1). In this case, a linear substrate is defined as having no branching on the adjacent residue.

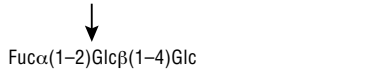
New Specificity

Specificity:

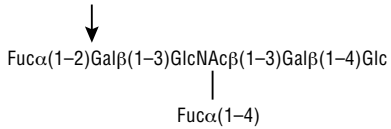
Note: p-nitrophenyl-α-L-fucopyranoside is NOT a substrate for this enzyme.

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

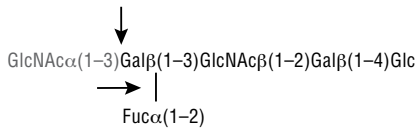
A. 0.1 nm/µl substrate, 1 hour incubation



B. 0.1 nm/µl substrate, 1 hour incubation



C. 0.05 nm/µl substrate, 1 hour incubation



D. 0.1 nm/µl substrate, 1 hour incubation

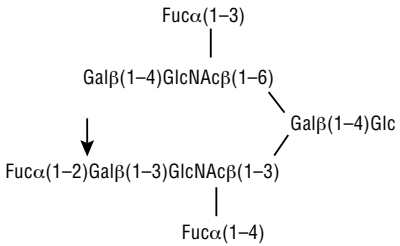


Figure 1: Detailed specificity of α1-2 Fucosidase. *Reactions (A), (B) and (D) contained 1X G4 reaction buffer and reactions (C) contained 1X G6 reaction buffer. All reactions contain 1X BSA In a total reaction volume of 10 µl, and all reactions were incubated at 37°C. All reactions contained 20 units of α1-2 Fucosidase. Reaction (C) also contained 20 units of α-N-Acetylgalactosaminidase (#P0734). In reaction (C), the branched α1-2 fucose is removed in the presence of both enzymes, but not by α1-2 Fucosidase alone.*

Source: *Xanthomonas manihotis*

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 0.1 mM Na_2EDTA.

Reagents Supplied with Enzyme:
10X G4 Reaction Buffer
100X BSA

Reaction Conditions: 1X G4 Reaction Buffer: 50 mM Sodium Citrate (pH 6.0 @ 25°C), 100 mM NaCl. Supplement with 100 µg/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 Fucα1-2Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

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

Molecular Weight: 70,000 daltons

Unit Definition Assay: Two fold dilutions of α1-2 Fucosidase are incubated with 1 nmol AMC-labeled substrate in 1X G4 Reaction Buffer, supplemented with 100 µg/ml BSA, in a 10 µl 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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Fuc α 1 – 2 R
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Detailed Specificity: Specificity can vary depending on incubation time and concentration of substrate (Fig 1).
A. 0.1 nm/µl substrate, 1 hour incubation
Fucα(1–2)Glcβ(1–4)Glc
B. 0.1 nm/µl substrate, 1 hour incubation
Fucα(1–2)Galβ(1–3)GlcNAcβ(1–3)Galβ(1–4)Glc
Fucα(1–4)
C. 0.05 nm/µl substrate, 1 hour incubation
GlcNAcα(1–3)Galβ(1–3)GlcNAcβ(1–2)Galβ(1–4)Glc
Fucα(1–2)
D. 0.1 nm/µl substrate, 1 hour incubation
Fucα(1–3)
Galβ(1–4)GlcNAcβ(1–6)
Galβ(1–4)Glc
Fucα(1–2)Galβ(1–3)GlcNAcβ(1–3)
Fucα(1–4)
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100X BSA
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(see other side)
CERTIFICATE OF ANALYSIS

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

Quality Controls

Glycosidase Assays: 100 units of α1-2 Fucosidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 µl 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:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-Fucosidase:
Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ 1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4GlcNAc-AMC ND

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ 1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

β-Mannosidase:
Manβ1-4Manβ1-4Man-AMC ND

Endo F_1, F_2, H:
Dansylated invertase high mannose. ND

Endo F_2, F_3:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 100 units of α1-2 Fucosidase with 0.2 nmol of a standard mixture of proteins in a 20 µl 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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β-N-Acetyl-glucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-Fucosidase:
Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ 1-4Glc-AMC ND

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β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4GlcNAc-AMC ND

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ 1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

β-Mannosidase:
Manβ1-4Manβ1-4Man-AMC ND

Endo F_1, F_2, H:
Dansylated invertase high mannose. ND

Endo F_2, F_3:
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