α1-2,4,6 Fucosidase

1-800-632-7799
info@neb.com
www.neb.com

P0748S 002150616061

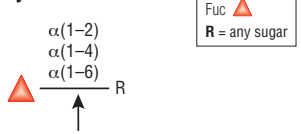
P0748S

400 units **8,000 U/ml** **Lot: 0021506**

Store at 4°C **Exp: 6/16**

Description: α1-2,4,6 Fucosidase is a broad specificity exoglycosidase that catalyzes the hydrolysis of α1-2, α1-4, and α1-6 linked L-fucopyranosyl residues from oligosaccharides. α1-2,4,6 Fucosidase cleaves α1-6 fucose residues more efficiently than other linkages, and has slight activity towards α1-3 fucose residues.

Specificity:



Source: Cloned from *bovine kidney* and expressed in *E. coli* (1).

Supplied in: 20 mM Tris-HCl (pH 7.5), 50 mM NaCl and 1 mM EDTA

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

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 α1-2Galα1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Specific Activity: 72,000 U/mg

Molecular Weight: 51,800 daltons

Unit Definition Assay: Two fold dilutions of α1-2,4,6 Fucosidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1 supplemented with 100 µglmL BSA in a 10 µl reaction. The reaction mix is incubated at 37°C for 1 hour. Separation of reaction products are visualized via thin layer chromatography (2).

Quality Assurance: No contaminating exoglycosidase or Endoglycosidase F1, F2 or F3 activity could be detected. No contaminating proteolytic activity could be detected.

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

Heat Inactivation: 8 units of enzyme were inactivated by incubation at 100°C for 10 minutes.

Reaction Protocol

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate. Typical reaction conditions are as follows:

1. Combine 1 µg of glycoprotein or 100 nM of oligosaccharide and H_2O (if necessary) to make an 8 µl total reaction volume.
2. Add 1 µl of 10X GlycoBuffer 1 and 1 µl of 10X BSA (diluted 1:10 from 100X concentration) to make a 10 µl total reaction volume.
3. Add 1 µl of α1-2,4,6 Fucosidase.
4. Incubate at 37°C for 1 hour.

Notes: Reactions may be scaled-up linearly to accommodate larger reaction volumes.

The amount of exoglycosidase enzyme required varies when different substrates are used. Start with 1–2 µl for 1µg of glycoprotein or 100 nM of oligosaccharide for one hour in a 10–25 µl reaction. If there is still undigested material, let the reaction go overnight.

(see other side)

CERTIFICATE OF ANALYSIS

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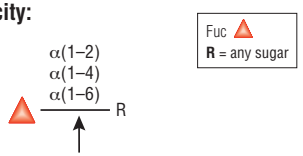

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(see other side)

CERTIFICATE OF ANALYSIS

α1-2,4,6 Fucosidase will partially cleave linear and branched α1-3 Fucose residues with low efficiency.

α1-2,4,6 Fucosidase will cleave branched α1-4 and α1-6 fucose residues but will not cleave branched α1-2 fucose residues. Longer incubation times (4 hours to overnight) may be needed for complex, branched oligosaccharide substrates.

Quality Controls

Glycosidase Assays: 80 units of α1-2,4,6 Fucosidase were incubated with 0.1 mM of flourescently-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.

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

β-N-Acetylglucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

β-N-Acetylgalactosaminidase:
GalNAcβ1-4Galβ1-4Glc-AMC ND

α-N-Acetylgalactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

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

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND
Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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

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

α-Glucosidase:
Glcα1-6Glcα1-4Glc-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

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 400 units of α1-2,4,6 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.

References:

1. Vainauskas, S., New England Biolabs, Inc. unpublished results.
2. Wong-Madden, S.T. and Landry, D. (1995) *Glycobiology* 5, 19–28.

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β-N-Acetylgalactosaminidase:
GalNAcβ1-4Galβ1-4Glc-AMC ND

α-N-Acetylgalactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

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

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND
Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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

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

α-Glucosidase:
Glcα1-6Glcα1-4Glc-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

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