α1-3,4 Fucosidase

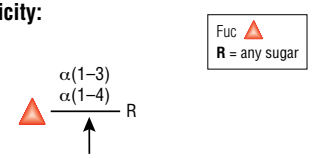
1-800-632-7799
info@neb.com
www.neb.com

P0769S

200 units	**4,000 U/ml**	**Lot: 0041508**
Store at 4°C		**Exp: 8/16**

Description: α1-3,4 Fucosidase, (also known as AMF) is a broad specificity exoglycosidase that catalyzes the hydrolysis of α1-3 and α1-4 linked fucose residues from oligosaccharides and glycoproteins.

Specificity:



Source: Cloned from the sweet almond tree (*Prunus dulcis*) and expressed in *Pichia pastoris* (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-fucose from 1 nmol of Galβ1-4GlcNAcβ1-3(Fucα1-3)Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Specific Activity: 75,000 U/mg

Molecular Weight: 56,000 daltons

Unit Definition Assay: Two fold dilutions of α1-3,4 Fucosidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1 supplemented with 1X 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 65°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 α1-3,4 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 1 µg 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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(see other side)

CERTIFICATE OF ANALYSIS

Higher enzyme concentrations and longer incubation times may be required for the removal of α1-3 and α1-4 fucose residues from complex *N*-glycans.

α1-3,4 Fucosidase can cleave the α1-3 fucose on the outer arm of *N*-linked glycans but is unable to remove the core α1-3 fucose.

Quality Controls

Glycosidase Assays: 16 units of α1-3,4 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 the substrate.

No other glycosidase activities were detected (ND) using 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
Galα1-6Galβ1-6Glcα1-2Fru-AMC ND

α-Fucosidase: Fucα1-2Galβ1-4Glc-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

Protease Assay: After incubation of 112 units of α1-3,4 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. Ganatra, M., 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
Galα1-6Galβ1-6Glcα1-2Fru-AMC ND

α-Fucosidase: Fucα1-2Galβ1-4Glc-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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2. Wong-Madden, S. T. and Landry, D. (1995) *Glycobiology* 5, 19–28.

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