

α 1-2,4,6 Fucosidase



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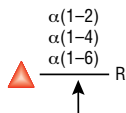
P0748S 002150216021

P0748S

400 units **8,000 U/ml** **Lot: 0021502**
Store at 4°C **Exp: 2/16**

Description: α 1-2,4,6 Fucosidase is a broad specificity exoglycosidase that catalyzes the hydrolysis of α 1-2, α 1-4, and α 1-6 linked L-fucopyranosyl residues from oligosaccharides. α 1-2,4,6 Fucosidase cleaves α 1-6 fucose residues more efficiently than other linkages, and has slight activity towards α 1-3 fucose residues.

Specificity:



Fuc
R = any sugar

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 α 1-2Gal α 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: 72,000 U/mg

Molecular Weight: 51,800 daltons

Unit Definition Assay: Two fold dilutions of α 1-2,4,6 Fucosidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1 supplemented with 100 μ g/mL BSA in a 10 μ l 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 μ g of glycoprotein or 100 nM of oligosaccharide and H₂O (if necessary) to make an 8 μ l total reaction volume.
2. Add 1 μ l of 10X GlycoBuffer 1 and 1 μ l of 10X BSA (diluted 1:10 from 100X concentration) to make a 10 μ l total reaction volume.
3. Add 1 μ l of α 1-2,4,6 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 μ l for 1 μ g of glycoprotein or 100 nM of oligosaccharide for one hour in a 10–25 μ l reaction. If there is still undigested material, let the reaction go overnight.

(see other side)

CERTIFICATE OF ANALYSIS

α 1-2,4,6 Fucosidase



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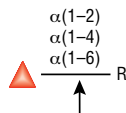
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(see other side)

CERTIFICATE OF ANALYSIS

α 1-2,4,6 Fucosidase will partially cleave linear and branched α 1-3 Fucose residues with low efficiency.

α 1-2,4,6 Fucosidase will cleave branched α 1-4 and α 1-6 fucose residues but will not cleave branched α 1-2 fucose residues. Longer incubation times (4 hours to overnight) may be needed for complex, branched oligosaccharide substrates.

Quality Controls

Glycosidase Assays: 80 units of α 1-2,4,6 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.

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

β -N-Acetylglucosaminidase:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

β -N-Acetylgalactosaminidase:
GalNAc β 1-4Gal β 1-4Glc-AMC ND

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β -N-Acetylgalactosaminidase:
GalNAc β 1-4Gal β 1-4Glc-AMC ND

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α -N-Acetylgalactosaminidase:
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

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

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal-AMC ND
Gal α 1-6Gal α 1-6Glc α 1-2Fru-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 ND
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

α -Glucosidase:
Glc α 1-6Glc α 1-4Glc-AMC ND

β -Xylosidase:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

α -N-Acetylgalactosaminidase:
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

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

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal-AMC ND
Gal α 1-6Gal α 1-6Glc α 1-2Fru-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 ND
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

α -Glucosidase:
Glc α 1-6Glc α 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

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Endo F₂, F₃:
Dansylated fibrinogen biantennary. ND

PNase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 400 units of α 1-2,4,6 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.

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

Endo F₁, F₂, H:
Dansylated invertase high mannose. ND

Endo F₂, F₃:
Dansylated fibrinogen biantennary. ND

PNase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 400 units of α 1-2,4,6 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.

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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