α1-2, 4, 6 Fucosidase

**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.

**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.

**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.

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

**Unit Definition Assay:**

1. Combine 1 µg of glycoprotein or 100 nM of particular substrate. Typical reaction conditions are as follows:

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. 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.

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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 fluoresecnated-label lopoligosaccharides 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**: GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND
- **α-Galactosidase**: Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND
- **α-Galactosidase**: Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC ND
- **α-Neuraminidase**: Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC ND
- **α-Mannosidase**: Manβ1-6Manβ1-6(Manβ1-3)Man-AMC ND
- **α-Glucosidase**: Glcβ1-6Glcβ1-4Glc-AMC ND
- **β-Xylosidase**: Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND
- **β-A-Galactosidase**: Galβ1-3Galβ1-4GlcNAc-AMC ND

### References:

3. ISO 9001:2000
4. ISO 14001:2004
5. ISO 13485:2003