**β-N-Acetyl-hexosaminidase**

**Description:** β-N-Acetyl-hexosaminidase is a recombinant protein fusion of β-N-Acetyl-hexosaminidase and maltose binding protein. It has identical activity to β-N-Acetyl-hexosaminidase and catalyzes the hydrolysis of terminal β-N-acetyl-galactosamine and glucosamine residues from oligosaccharides.

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave > 95% of the terminal β-N-acetyl-galactosamine from 1 nmol of GalNAcβ1-4Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

**Unit Definition Assay:** Two fold dilutions of β-N-Acetyl-hexosaminidase, are incubated with 1 nmol AMC-labeled substrate in 1X G2 Reaction Buffer 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 (3).

**Specific Activity:** ~ 10,000 units/mg

**Molecular Weight:** 100,000 daltons

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

**Quality Controls**

**Glycosidase Assays:**
50 units of β-N-Acetyl-hexosaminidase, 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.

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

- **α-Fucosidase:** Fucx1-2Galβ1-4Glc-AMC Galβ1-4 (Fucx1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND
- **β-Galactosidase:** Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND
- **α-Galactosidase:** Galα1-3Galβ1-4Galc1-3Gal-AMC ND
- **α-Neuraminidase:** Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC ND
- **α-Mannosidase:** Manα1-3Manβ1-4GlcNAc-AMC Manα1-6Manα1-6(Manα1-3)Man-AMC ND
- **β-Glucosidase:** Glcβ1-4Glcβ1-4Glc-AMC ND

(See other side)
β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC  ND

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

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

Endo F₃, F₄:  
Dansylated fibrinogen biantennary.  ND

PNGase F:  
Fluoresceinated fetuin triantennary.  ND

Protease Assay: After incubation of 50 units of β-N-Acetyl-hexosaminidase, 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: Non-branched oligosaccharides only.

References: