α-N-Acetyl-galactosaminidase

**Source:** Cloned from *Chryseobacterium meningosepticum* and expressed in *E. coli* at NEB (1).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 0.1 mM EDTA.

**Reagents Supplied with Enzyme:**
10X G7 Reaction Buffer, 100X BSA

**Reaction Conditions:**
1X G7 Reaction Buffer:
50 mM Sodium Phosphate (pH 7.5 @ 25°C), supplement with 100 µg/ml BSA. Incubate at 37°C.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave > 95% of the (GalNAcα1-3)(Fucα1-4Glc-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 α-N-Acetyl-galactosaminidase are incubated with 1 nmol AMC-labeled substrate in 1X G1 Reaction Buffer, supplemented with 100 µg/ml BSA, 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 (2).

**Specific Activity:** 20,000 units/mg

**Molecular Weight:** 47,000 daltons.

**Quality Assurance:** No other glycosidase or proteolytic activity could be detected.

**Quality Controls**

Glycosidase Assays: 20 units of α-N-Acetyl-galactosaminidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C (see list below). The reaction products were analyzed by TLC for digestion of substrate (3).

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

(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 20 units of α-N-Acetyl-galactosaminidase with 0.2 nmol of a standard mixture of proteins, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Note: Recommended storage temperature has changed to –20°C. Avoid repeated freeze/thaw cycles.

References:

U.S. Patent No. 6,458,573 and 6,423,525