**Endo - alpha - N - acetylgalactosaminidase**  
(from *Alcarigenes* sp.)  
**EC 3.2.1.97**

**SYSTEMATIC NAME:**
- D - Galactosyl - N - acetamidodeoxy - alpha - D - galactoside  
- D - Galactosyl - N - acetamidodeoxy - D - galactohydrolase  

Endo - alpha - N - acetylgalactosaminidase was purified from the culture fluid of *Alcarigenes* sp. F - 1906. This enzyme acts on sugar chains called O - glycan and hydrolyses N - acetylgalactosamin - Ser (or Thr) linkage shown below. The enzyme doesn't act on sugar chains having sialic acid.

**SPECIFICATIONS:**
- **Appearance**  
  Solution in 10mM K - phosphate buffer, pH 6.0 containing 40% glycerin.
- **Activity**  
  Approx. 3U/mg
- **Contaminants**  
  The enzyme preparation has no detectable activity of various exo - glycosidases.
- **Purity**  
  Homogeneous by disc - PAGE
- **Optimum pH, temp.**  
  pH4.5, 40°C
- **pH stability**  
  pH4.5-7.0 (30°C, 1hr)
- **Thermostability**  
  Up to 30°C (pH6.0, 10min)
- **Km value**  
  3.7mM (asialofetuin as substrate)
- **Molecular weight**  
  Approx. 160,000 (gel filtration or SDS - PAGE)
- **Inhibitor**  
  The enzyme activity isn't inhibited by SH reagent or galactose.

**ASSAY FOR ENZYME ACTIVITY:**

**Unit Definition**
One unit of enzyme catalyzes the release of 1 micro mole of Gal-GalNAc from asialofetuin per minute at 37°C, pH4.5.

**Method**

**Reaction mixture**
- Substratel: 0.4mM asialofetuin 40 micro l  
- Buffer: 0.2M citrate buffer pH4.5 10 micro l  
- Enzyme: Suitably diluted enzyme (0.2-1.8mU) 30 micro l

Total volume 80 micro l

**Procedure**

After incubation at 37°C for 10minutes, the reaction is terminated by adding 100 micro l of 0.8M borate buffer pH9.1. The amount of reaction product is determined by Reissing method using 2.65 x 10⁻³ as molecular extinction coefficient of Gal - GalNAc.
Calculation

Enzyme unit = [amount of Gal - GalNAc] (micro mole) x 1/10

STORAGE:

Please keep at -20°C when received. At -20°C no appreciable decrease was observed in its activity at least for a year.

REFERENCES:

2) J. Biol. Chem., 217, 959 (1955)

NOTE:

For laboratory use only. Not for drug, household or other uses.