



## O-Glycosidase

Endo-*alpha*-N-Acetylgalactosaminidase

### Source

recombinant *Streptococcus pneumoniae* in *E.Coli*

EC 3.2.1.97

Catalog Number E-G001

Certification of Analysis Lot Number 901.1A

### Contents

1 vial: O-Glycosidase  
75 mU in 60  $\mu$ l

1 vial: 5x Reaction buffer  
250 mM sodium phosphate, pH 5.0

**Specific Activity** >12 U/mg

**Activity** 1.25 U/ml

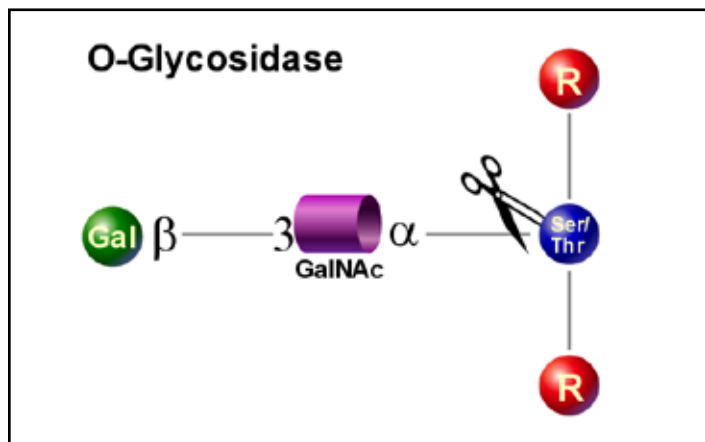
### Specificity

Cleaves only unsubstituted Gal- $\beta$ (1-3)GalNAc- $\alpha$  disaccharides attached to the serine or threonine residues of glycoproteins or glycopeptides. Substitutions such as sialic acid, galactose, fucose or N-acetylglucosamine must first be removed with the appropriate exoglycosidase prior to treatment with O-Glycosidase.

At minimum, a sialidase such as Sialidase Au (Alpha-2-3,6,8,9), part number E-S001, is almost always required to remove sialic acids

### Formulation

The enzyme is provided as a sterile-filtered solution in 50 mM sodium phosphate (pH 7.5).



### Properties

molecular  $\sim$ 180,000 daltons

pH optimum: 5, active over the range 5-7.

### Storage

Store enzyme at 4°C. Do not freeze.

### Specific Activity

One unit of O-Glycosidase is defined as the amount of enzyme required to produce 1  $\mu$ mole of *p*-nitrophenol (*p*NP) in 1 minute at 37°C, pH 5.0 from *p*-nitrophenyl-2-acetamido-2-deoxy-3-O-(*beta*-D-galactopyranosyl)-*alpha*-D-galactopyranoside.

### Purity

O-Glycosidase is tested for contaminating protease as follows: 10  $\mu$ g of denatured BSA is incubated at 37°C for 24 hours with 2  $\mu$ l of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation. The production host strain has been extensively tested and does not produce any detectable glycosidases.

**E-G001 O-Glycosidase**  
**Specifications - Protocol**

**Directions for use**

1. Add up to 100 µg of glycoprotein to tube.
2. Add de-ionized water to a total of 13 µl.
3. Add 4 µl 5x Reaction Buffer 5.0.
4. Add 1 µl Sialidase AU (E-S001)
5. Add 2 µl O-Glycosidase.
6. Incubate at 37°C for 1 hour.

Cleavage may be monitored by SDS-PAGE if the size differential between native and de-O-glycosylated protein is sufficient for detection.

**References**

Bhavanandan, V.P. , J. Umemoto and E.A. Davidson. Characterization of an endo-alpha-N-acetylgalactosaminidase from *Diplococcus pneumoniae*. **Biochem Biophys** **70**:738-745 (1976).

Fan, J. Q., K. Yamamoto, H. Kumagai and T. Tochikura. Induction and efficient purification of endo-alpha-N-acetyl-D-galactosaminidase from *Alcaligenes* sp. **Agric Biol Chem** **54**:233-234 (1990).

Glasgow, L R., J. C. Paulson and R. L. Hill. Systematic purification of five glycosidases from *Streptococcus pneumoniae*. **J Biol Chem** **252**:8615-8623 (1977).

Iwase, H. and K. Hotta. Release of O-linked glycoprotein glycans by endo-alpha-N-acetyl-D-galactosaminidase. **Methods Mol Biol** **14**:151-159 (1993).

Unemoto, J., V. P. Bhavanandan and E. A. Davidson. Purification and properties of an endo-alpha-N-acetyl-D-galactosaminidase from *Diplococcus pneumoniae*. **J Biol Chem** **252**:8609-8614 (1977).

**Warranties and liabilities**

QA-Bio warrants that the above product conforms to the attached analytical documents. Should the product fail for reasons other than through misuse QA-Bio will, at its option, replace free of charge or refund the purchase price. This warranty is exclusive and QA-Bio makes no other warrants, expressed or implied, including any implied conditions or warranties of merchantability or fitness for any particular purpose.

QA-Bio shall not be liable for any incidental, consequential or contingent damages.

This product is intended for *in vitro* research only.

*updated January 2, 2009*