



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 μ l

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

Specific Activity >12 U/mg

Activity 1.25 U/ml

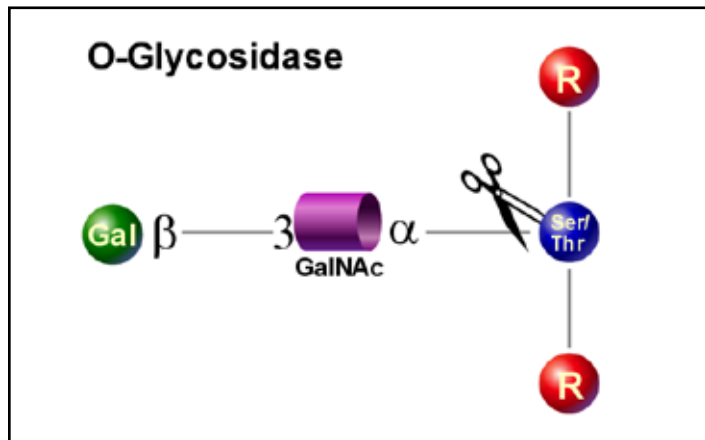
Specificity

Cleaves only unsubstituted Gal- β (1-3)GalNAc- α 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 μ 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 μ g of denatured BSA is incubated at 37°C for 24 hours with 2 μ 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

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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).

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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).

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