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 571.1A

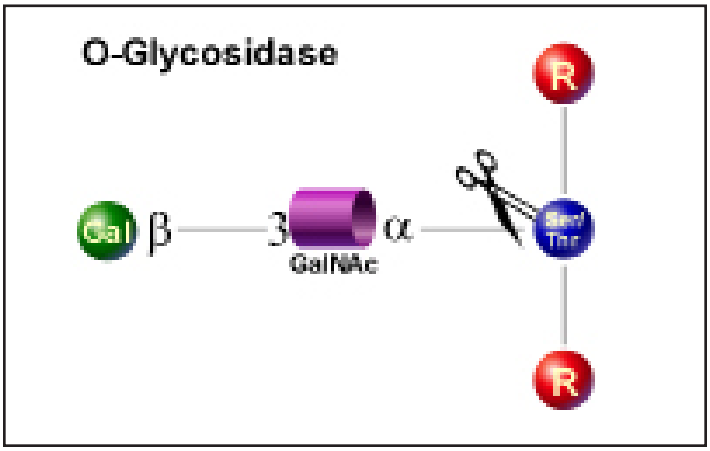
Contents
1 vial: O-Glycosidase
75 mU in 60 µl
1 vial: 5x Reaction buffer
250 mM sodium phosphate, pH 5.0

Specific Activity >12 U/mg
Activity 1.25 U/ml

Specificity
Cleaves only unsubstituted Gal-ß(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 sialadase 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 m*M* sodium phosphate (pH 7.5).



Properties
molecular ~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 µmole 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 µg of denatured BSA is incubated at 37°C for 24 hours with 2 µl 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

QA-Bio
www.QA-Bio.com
info@QA-Bio.com

866-384-2272
760-565-3563
fax 760-262-3139

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).

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).

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