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O-Glycosidase

O-Glycosidase (O-glycopeptide endo-D-galactosyl-N-acetyl-α-galactosaminohydrolase, EC 3.2.1.97) cleaves only unsubstituted Galβ(1-3)GalNAcα 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 neuraminidase such as α(2-3,6,8,9) neuraminidase is almost always required to remove sialic acid.

There is no activity on α-GalNAc linked either to protein or carbohydrate. Although limited by its strict specificity, O-Glycosidase, in conjunction with other exoglycosidases, is still the method of choice for removing O-linked sugars from glycoproteins. The protein remains intact, as does the disaccharide.

O-Glycosidase is isolated from a clone of *Streptococcus pneumonia* (formerly *Diplococcus pneumonia*).

O-Glycosidase is useful for:

- Determining O-glycosylation in proteins
- Studying the effects of O-glycosylation in binding and antigenic studies
- Removing O-linked sugars for X-Ray crystallography and protein sequencing

Specifications

Activity
≥16 U/mg, ≥17 U/mL

Storage
Store at 4°C. Do not freeze

Formulation
Enzyme is provided as a sterile solution in 50 *mM* Sodium Phosphate pH 7.5.

Stability
Stable at least 12 months when stored properly. Several days exposure to ambient temperature will not reduce activity.

Product Description

Molecular Weight
~ 180,000 Daltons

Purity
O-Glycosidase is tested for contaminating protease as follows; 10 µg of denatured BSA is incubated for 24 hours at 37°C 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.

Specificity:
Serine- or threonine-linked unsubstituted Galβ(1-3) GalNAcα.

pH Range:
Optimum: pH 5 Range: pH 5 - 7

The supplied buffer concentrate provides the optimal pH for enzyme activity with the standard substrate. If glycosidase treatment is performed at suboptimal pH because of glycoprotein solubility or activity requirements, expect some diminution in enzyme activity.

Assay

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 from *p*-nitrophenyl-2-acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-α-D-galactopyranoside.

Reagents

- 5X PNGase buffer 5- 250 *mM* sodium phosphate pH 5

Note: O-Glycosidase cleaves methylumbelliferyl-α-D-N-acetylgalactosamide (but not pNP-α-D-N-acetyl-galactosamide) at 0.1% the rate of *p*-nitrophenyl-2-acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-α-D-galactopyranoside.

Suggestions for Use

Procedure for Deglycosylation

1. Add up to 100 µg of glycoprotein to tube.
2. Add water to 13 µL and 4 µL 5X Reaction Buffer 5
3. Add 1 µL α(2-3,6,8,9) neuraminidase
4. Add 2 µL O-Glycosidase
5. 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

1. Bhavanandan, V.P. , J. Umemoto and E.A. Davidson. Characterization of an endo-alpha-N-acetylgalactosaminidase from Diplococcus pneumonia. Biochem Biophys 70:738-745 (1976).
2. Fan, J. Q., K. Yamamoto, H. Kumagai and T. Tochikura. Induction and efficient purification of endo-alpha-Nacetyl-D-galactosaminidase from Alcaligenes sp. Agric Biol Chem 54:233-234 (1990).
3. Glasgow, L R., J. C. Paulson and R. L. Hill. Systematic purification of five glycosidases from Streptococcus pneumonia. J. Biol Chem 252:8615-8623 (1977). Iwase, H. and K. Hotta. Release of O-linked glycoprotein glycans by endo-alpha-N-acetyl-Dgalactosaminidase. Methods Mol Biol 14:151-159 (1993).
4. Unemoto, J., V. P. Bhavanandan and E. A. Davidson. Purification and properties of an endo-alpha-Nacetyl-D-galactosaminidase from Diplococcus pneumonia. J. Biol Chem. 252:8609-8614 (1977).

Order Information

Catalog No.	Product Description	Package Size	Temp. °C
100686-1	**O-Glycosidase** *(Streptococcus pneumonia* recombinant*)*	60 µL	+4

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