Selectin Biosciences Inc.

Endo-O-Glycosidase

Endo-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, fucose, galactose or N-acetylglucosamine must first be removed with the appropriate exoglycosidase prior to treatment with Endo-O-Glycosidase. At minimum, a sialidase such as GE 23, from Arthrobacter ureafaciens is almost always required to remove sialic acid.

There is minimal activity on α-GalNAc and other substituted core 2 glycans. Although limited by its strict specificity, Endo-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.

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

Endo-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

Product Code: GE 43

Specifications

Activity: ≥ 12 U/mg, ≥ 1.25 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. Active for at lease 5 days under reaction conditions.

Product Description

Molecular weight: ~180,000 Daltons

Purity: Endo-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 Endo-O-Glycosidase is defined as the amount of enzyme required to produce 1 μmole of p-nitrophenol (pNP) in 1 minute at

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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: Endo-O-Glycosidase cleaves methylumbelliferyl-α-D-N-acetylgalactosamide (but not pNP-α-D-N-acetylgalactosamide) at 0.1% the rate of -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 Alpha (2-3, 6, 8, 9) neuraminidase
4. Add 2 µL Endo-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

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- 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 pneumonia. **J. Biol Chem 252:**8615-8623 (1977).
- 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).

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This product is intended for in vitro research only.

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