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ProductInformation

O-Glycosidase
from *Streptococcus pneumoniae*, recombinant
expressed in *E. coli*

Product Number **G 1163**
Storage Temperature 2–8 °C

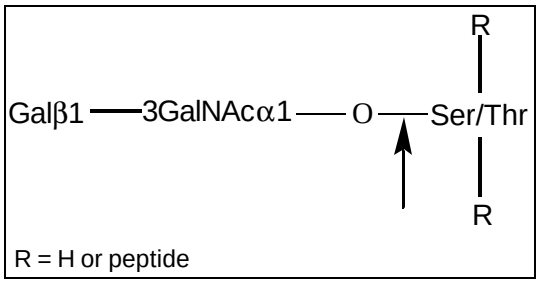
CAS# 9032-92-2
EC 3.2.1.97
Synonyms: Endo-α-N-acetylgalactosaminidase; Endo-α-acetylgalactosaminidase; O-Glycanase

Product Description
One of the distinguishing features of the proteome in eurkaryotic cells is that most proteins are subject to post-translational modification, of which glycosylation is the most common form. It is estimated that more than half of all proteins are glycoproteins. Two major classes of oligosaccharides (glycans) may be attached to proteins. N-linked glycans are attached to the amide side chain of Asn residues, which form part of the consensus sequence AsnXaaSer/Thr, while O-linked glycans may be added to the hydroxyl side chain of Ser or Thr residues. The core structure and composition of O-linked glycans is different than that of N-linked glycans. The most common core structure of O-glycans consists of the disaccharide Gal-β-(1→3)-GalNAc, α-linked to the hydroxyl of Ser or Thr residues.

Removal of O-glycans has been used for:
- Detection of O-glycosylation in glycoproteins[1]
- Glycoprotein deglycosylation[2]
- Structural analysis of O-glycans[3]
- Epitope and binding site analysis[4]
- Bioactivity in O-glycans
- Analysis of biosynthesis of glycoproteins[5]

This product is a highly specifc enzyme, which hydrolyzes the N-acetylgalactosamine glycosidic linkage, liberating the core disaccharide from the serine or threonine residue that is either unsubstituted or present in glycoproteins or glycopeptides.[6]

The enzyme is specific for α-GalNAc linkages, but it has no apparent preference for serine over threonine-linked residues.[7] Absence of the galactose residue or the acetamido group on the innermost N-acetyl-galactosamine residue also prevents cleavage of the O-glycan linkage.[7,8]



Any modification of the core structure can block the action of O-glycosidase. If the O-glycan structure is larger than the core structure, for example substituted with N-acetylglucosamine, N-acetylgalactosamine, sialic acid, or fucose, O-glycosidase will not cleave the GalNAc to Ser/Thr linkage. In such cases the additional monosaccharides must be sequentially hydrolyzed by a series of exoglycosidases, until only the Gal-β-(1→3)-GalNAc core remains. O-Glycosidase can then remove the core structure intact with no modification of the serine or threonine residue. The enzyme is reported to cleave >90% of the disaccharide core from antifreeze glycoprotein and virtually 100% from porcine submaxillary mucin when used in conjunction with α-fucosidase, α-N-acetylgalactosaminidase, and sialidase.

Molecular weight: ~180 kDa

Isoelectric point (pI):[9] ~8.0

Optimal pH: 5.0 - 6.0.

Inhibition:[7]
1 mM EDTA (63%)
1 mM p-chloromercuribenzenesulfonic acid (60%)
1 mM Mn^{2+} (44%)
1 mM Zn^{2+} ions (66%)

Chloride is also reported to inhibit the enzyme, therefore, Tris HCl buffers should be avoided where possible.[8]

Components
O-glycosidase (Product No. G 1163) – The enzyme is supplied in 50 mM sodium phosphate, pH 7.5.

Unit Definition: One unit will hydrolyze 1 µmole of PNP Galacto-N-bioside [Product No. N 3016, Gal-β(1-3)-GalNAc-α(1-O)-p-nitrophenyl] per minute at 37 °C at pH 5.0.

The recombinant host strain produces no detectable glycosidase activity. Protease activity was also not detected.

5X Reaction Buffer (Product No. E 5879) – 250 mM sodium phosphate, pH 5.0

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
It is recommended to store the product at 2–8 °C. Both the enzyme and buffer are stable for 1 year if stored unopened at 2–8 °C. Once reconstituted the enzyme solution should be stored at 2–8 °C and used within 7 days. Diluted buffer should be stored at 2–8 °C and used within 7 days.

Procedure
1. Add up to 100 µg of glycoprotein to a microcentrifuge tube.
2. Add deionized water to a total volume of 13 µl.
3. Add 4 µl of 5X Reaction Buffer (Product No. E 5879).
4. If sialylated glycans are present, then add 1 µl of broad-spectrum sialidase (Product No. N 8271, see note).
5. Incubate at 37 °C for 1 hour.
6. Add 2 µl of O-Glycosidase (Product No. G 1163).
7. Incubate at 37 °C for 1–3 hours.

Note: Removal of O-glycans, which are more complex than the core disaccharide, may require prior addition of exoglycosidases:
sialidase (Product No. N 8271)
α-fucosidase (Product No. F 5884)
β-hexosaminidase (Product No. A 7708)
α-N-acetylgalactosaminidase (Product No. A 9763)

References

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