

# O-GLYCANASE™

## (Endo- $\alpha$ -N-Acetylgalactosaminidase)

### SPECIFICATIONS

**Product Code:** GK80090  
**Specific Activity:**  $\geq 12$  U/mg  
**Activity:**  $\geq 1.25$  U/ml  
 Shipped on cold pack for next day delivery.  
 Store at 4°C. **DO NOT FREEZE.**  
**Formulation:** A sterile-filtered solution in 20 mM Tris-HCl, 25 mM NaCl (pH 7.5)  
**Stability:** Stable at least 12 months when stored properly.

Glyko® sequencing-grade O-Glycanase™ (O-glycopeptide endo-D-galactosyl-N-acetyl- $\alpha$ -galactosaminohydrolase) is isolated from a strain of *E. coli* expressing a cloned gene from *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*).

Glyko® O-Glycanase is useful for:

- Detection of O-glycosylation in proteins<sup>1,2</sup>
- Cancer studies and identification of aberrant O-glycosylation<sup>3,4,5</sup>
- Characterization of therapeutic recombinant proteins<sup>6</sup>
- Pathophysiology studies involving O-glycans<sup>7,8</sup>

### CHARACTERISTICS

**Supplied Reagents** (research pack only)

- WS0059 5x Reaction Buffer 5.0 (250 mM sodium phosphate, pH 5.0)

**Purity:** Glyko® O-Glycanase™ is free of contaminating endo- and exoglycosidase activity. No protease activity was detectable after incubation of the enzyme with 0.2 mg resorufin-labeled casein for ~18 hours at 37°C according to the method described by Twining (1984).

The production host strain has been extensively tested and does not produce any detectable glycosidases.

**Specificity:** Glyko® O-Glycanase™ cleaves Gal  $\beta$ (1-3) GalNAc  $\alpha$ - as an intact disaccharide unit from serine or threonine residues of glycoproteins or glycopeptides. This disaccharide is the defining structural component for Core 1 type O-linked glycans, see Figure 1. Cleavage of the glycosidic bond is between the GalNAc residue, in the alpha configuration, and the hydroxyl moiety of the amino acid side chain of the polypeptide. Substitutions of the disaccharide core with sialic acid, or a lactosaminic repeating unit of galactose-N-acetyl glucosamine of fucose, will block hydrolysis and prevent the liberation of the oligosaccharide from the protein

In order to expose the Core I type structure so that it is susceptible to the O-Glycanase™ action, extended oligosaccharides must first be treated with glycosidases, such as Glyko® Sialidase A™/NANase III (GK80040), or in

addition, treatment with a combination of Glyko®  $\beta(1-4)$ -galactosidase and Glyko®  $\beta$ -N-Acetylhexosaminidase/Hexase I (available together as GK80115), Figure 1.

The enzyme has no activity on single  $\alpha$ -GlcNAc linked either to protein or carbohydrate. With the synthetic substrate analog, Gal  $\beta(1-3)$  GalNAc-*p*-nitrophenyl glycosidase, a  $K_m$  value of  $\sim 200 \mu M$  was obtained. Interestingly, the enzyme is similar to other glycohydrolases and has been reported to have 'trans' glycosidase activity. Cleavage of the disaccharide unit is mediated by the formation of a covalent enzyme intermediate. The enzyme-bound glycan can be transferred to a number of hydroxylated acceptor molecules instead of displacement with water.

**Molecular Weight:**  $\sim 180,000$  daltons

**pH Range:**

Optimum: pH 5.0

Range: pH 5.0 - 6.0

**Properties:** The enzyme is inactivated with sulfhydryl reagents such as *p*-chloro-mercuribenzenesulfonic acid and transition metals such as  $Mn^{2+}$  or  $Zn^{2+}$ . The enzyme is also inhibited with 1 mM EDTA.

In a highly purified form, O-Glycanase® adsorbs to glass surfaces and is inactivated or gives variable activities. Assays with purified substrates should be carried out in polypropylene vessels, and transfer of the enzyme solutions with glass pipettes should be avoided.

The purified enzyme, as formulated, is stable at 4°C but about 30% of its activity is lost with a single freeze-thaw cycle. The enzyme activity is not significantly affected if the material is stored at room temperature for 24 hours.

The optimum buffer for enzyme activity with

the standard substrate is 50 mM sodium phosphate (pH 5.0). 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 Glyko® O-Glycanase™ is defined as the amount of enzyme required to produce 1  $\mu$ mole of *p*-nitrophenol (*p*NP) in 1 minute at 37°C, pH 5.5, from Gal  $\beta(1-3)$  GalNAc  $\alpha$  1-*p*NP.

**Additional Reagents** (not supplied)

- 250  $\mu M$  *p*-nitrophenyl-2-acetamido-2-deoxy-3-O-( $\beta$ -D-galactopyranosyl)- $\alpha$ -D-galactopyranoside (Sigma N3016) in 50 mM sodium phosphate (pH 5.0)
- 1 M sodium carbonate

## SUGGESTIONS FOR USE

**Procedure for Deglycosylation of a native protein:**

For a 20  $\mu$ l digestion volume,

1. Isolate glycoprotein and lyophilize about 100  $\mu$ g in a polypropylene tube.
2. Add 13  $\mu$ l of de-ionized water. If the protein is already in solution, add enough water to bring the total volume to about 13  $\mu$ l.
3. Add 4  $\mu$ l 5x Reaction Buffer 5.0.
4. Add 1  $\mu$ l Glyko® Sialidase A™/NANase III\* (GK80040).
5. Add 2  $\mu$ l O-Glycanase™.
6. Incubate at 37°C for 1 hour.

\*If the glycan structure is anticipated to have lactosaminic repeat, the enzymes in the PRO-LINK Extender™ Kit for Complex O-Linked Glycans (GK80115) may be added along with sialidase at this point.

For most proteins, although certainly not all, the contribution of O-linked structures is only a small percentage of the total molecular weight of the protein. Therefore it may be difficult to monitor deglycosylation using SDS-PAGE as the change in molecular weight between the glycosylated and non-glycosylated form of the protein may not be resolved sufficiently by this technique.

## REFERENCES

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Figure 1:

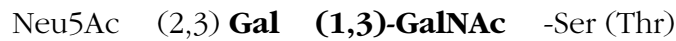
Core 1 type Structure of O-linked Glycans

**Core I**

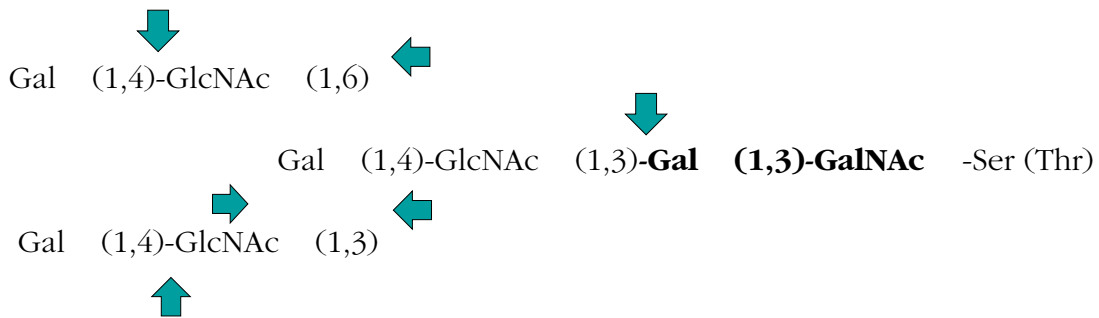


*O-Glycanase™ cleaves here*

Other Core I type O-linked Glycans



*Pretreat Glycan with Sialidase A™ to expose Core prior to treating with O-Glycanase™*



*Pretreat glycan or glycoprotein with hexosaminidase and  $\beta$ -galactosidase (PRO-LINK Extender, GK80115) to expose the Core, then incubate with O-Glycanase™.*



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