PROZOMIX
Recombinant Enzymes and Proteins

O-GlcNAc (*specific*) Deglycosylation Kit

Cat. No. PRO-K0001
20 x 50 µL reactions
Version 2010-1

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Introduction:

Serine- and threonine-linked *O*-GlcNAc post-translational modifications are receiving ever greater research attention due to their emerging ubiquitous occurrence on nuclear and cytoplasmic proteins such as oncogene proteins, heat shock proteins, nuclear pore proteins, viral proteins, and cytoskeletal proteins. The *O*-GlcNAc modification can impart functional changes in target proteins and affect transcription, translation, and signalling, and is often observed competing for phosphorylation sites.[1] To date the *O*-GlcNAc modification has been linked with the modulation of proteasome function, insulin resistance, cell cycle control, neutrophil function, and even Alzheimer's disease.[2] From an applied perspective, ever increasing attention is being paid to biopharma protein product homogeneity, where glycoprotein modifications can be responsible for significant and unpredictable heterogeneity.

Existing techniques based on hexosaminidase do exist for *O*-GlcNAc removal from glycoproteins. However, these methods are not specific for *O*-GlcNAc, and lead to the removal of both N-acetyl-β-D-glucosamine and N-acetyl-β-D-galactosamine from a number of linkage types. Prozomix thus developed this novel kit, that employs an engineered *O*-GlcNAcase, which enables simple and specific removal of *O*-GlcNAc.

Key product features:

- specific for *O*-GlcNAc (i.e. no activity against any GalNAc based substrate)
- complete removal of *O*-GlcNAc
- *O*-GlcNAcase employed:
  - has neutral pH optimum
  - is very stable (> 10 days at 37°C in deglycosylation reaction conditions)
  - can be freeze-thawed > 10 times after reconstitution
  - can be removed from reaction when deglycosylation is complete

Kit components and their preparation:

NOTE: this kit is shipped at room temperature, but should be stored at -20°C.

1. **0.5 M Tris/HCl buffer, pH 7.5, containing 100 mM imidazole**
   1 x 1.0 mL

Preparation: ready to use
Storage: store at -20°C

2. **0.5 M Sodium phosphate buffer, pH 7.0, containing 100 mM imidazole**
   1 x 1.0 mL

Preparation: ready to use
Storage: store at -20°C

3. ***O*-GlcNAcase**
   1 x vial of 2.69 mg (freeze-dried powder)

Preparation: reconstitute by addition of 0.25 mL H_2O before use (the enzyme will dissolve immediately and be at a concentration of 10.78 mg/mL (59.9 U/mL) in 50 mM Tris/HCl buffer, pH 7.5)
Storage: before reconstitution, store at -20°C. After reconstitution, store at -20°C (there is no need to freeze in aliquots, as this enzyme solution can be freeze-thawed > 10 x without loss of activity)

4. ***O*-GlcNAcase binding resin in 25 % ethanol**
   2 x 1.0 mL

Preparation: re-suspend thoroughly by flicking/inverting immediately prior to use
Storage: store at -20°C

5. **Mini-spin columns**
   x 5 (should it be required, additional columns can be purchased from Prozomix using product code PRO-KC0001-5)

Preparation: ready to use
Storage: store with other kit components at -20°C

Protocol:

1. *O*-GlcNAc (*specific*) deglycosylation reaction:

Component	Volume
Glycoprotein sample (at ~ 2 mg/mL)	35 μL
*Bottle 1 or 2 (buffer)	5 μL
**Bottle 3 (*O*-GlcNAcase)	10 μL
total	**50 μL**

2. Incubate reaction at 37°C for 2** days.

*Depending on the application, either Tris/HCl buffer (Bottle 1) or sodium phosphate buffer (Bottle 2) can be used.

**The deglycosylation reaction conditions described above should be considered strictly experimental, and are designed to afford complete deglycosylation of most samples. It could be, however, that either less time, or less *O*-GlcNAcase (Bottle 3), or even both, are actually required with a particular sample. Should it be desired, the incubation time can be extended up to 10 days at 37°C, as the *O*-GlcNAcase employed is very stable under the deglycosylation reaction conditions. Additionally, if less enzyme is required, sufficient extra buffer is provided in the kit to enable significantly more reactions than stated (20 x 50 μL), to be performed. Should the *O*-GlcNAcase be the only component used from the kit in your application(s), this individual component can be purchased more cost-effectively from Prozomix, using product code PRO-KC0001-3.

3. *O*-GlcNAcase removal step (***optional):

NOTE: the *O*-GlcNAcase removal procedure is not compatible with glycoprotein samples that contain polyhistidine purification tags.

a. Re-suspend the *O*-GlcNAcase binding resin (Bottle 4) thoroughly by flicking/inverting and pipette 100 μL into a mini-spin column (Component 5).
b. Place the mini-spin column into a new 1.5 mL centrifuge tube and spin for 1 min in a micro-centrifuge at 2000 rpm.
c. Add 125 μL of H_2O to the mini-spin column and centrifuge for 1 min at 2000 rpm.
d. Empty the 1.5 mL centrifuge tube and repeat step c twice.
e. Place the mini-spin column into a new 1.5 mL centrifuge tube (to collect the glycoprotein sample) and add the 50 μL deglycosylation reaction.
f. Mix by gentle flicking of the tube (but sufficient to fully resuspend the O-GlcNAcase binding resin).
g. Incubate for 2 min at room temperature.
h. Centrifuge the mini-spin column assemblage for 1 min at 2000 rpm.
i. Add 50 μL of H_2O to the mini-spin column and centrifuge the mini-spin column assemblage again for 1 min at 2000 rpm.
j. Recover the glycoprotein sample from the 1.5 mL centrifuge tube (the volume recovered will be ~ 100 μL), and approx. 98 % of the O-GlcNAcase will have been removed.

***In order to elicit complete deglycosylation, the reaction conditions contain a high final *O*-GlcNAcase concentration of ~ 2 mg/mL. Should it be desired to remove the *O*-GlcNAcase after the reaction is complete, this can be achieved as described above.

References:

1. Whelan SA, Hart GW (2003) Proteomic approaches to analyze the dynamic relationships between nucleocytoplasmic protein glycosylation and phosphorylation. *Circ Res.* **93**: 1047–1058.

2. Liu F, Iqbal K, Grundke-Iqbal I, Hart GW, Gong C-X (2004) *O*-GlcNAcylation regulates phosphorylation of tau: A mechanism involved in Alzheimer's disease. *Proc Natl Acad Sci U S A* **101**: 10804–10809.

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