

## Active Recombinant O-Glycoprotease, His tagged

**Cat. No.** O-Glycoprotease-13    **Lot. No.** (See product label)

### SPECIFICATION

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| <b>Product Overview</b> | O-glycoprotease is expressed in E.coli. This enzyme contains a 6×His tag, which allows for convenient removal after the reaction.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Source</b>           | E.coli                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Description</b>      | It is a highly specific glycoprotease that can specifically recognize the serine or threonine residues of mucin-type O-linked glycans (whether sialylated or not) and cleave at their N-termini. It can generate O-glycopeptides suitable for mass spectrometry analysis.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Purity</b>           | ≥ 95% by SDS-PAGE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Advantages</b>       | 1. Wide adaptability: It is active against O-glycoproteins with or without sialic acid, and shows higher activity on desialylated O-glycoproteins. It can effectively cleave glycoproteins without the need for neuraminidase treatment; 2. High purity: It is free from contamination by other proteases and has no other endo- and exo- glycosidase activities, with a purity of ≥ 95%; 3. High stability: Each batch of O-glycoprotease undergoes strict quality control to ensure batch-to-batch stability of the product; 4. Compatible with HPLC/MS: It does not contain glycerol, which helps to achieve optimal results in HPLC and mass spectrometry analyses; 5. Easy removal: It contains a 6×His-Tag and can be easily removed from the reaction system using nickel-affinity resin. |
| <b>Protein Content</b>  | ≥ 1 U/μL                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

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| <b>Unit definition</b> | One unit is defined as the amount of the enzyme required to cleave more than 90% of 2 $\mu$ M O-glycopeptide in a 20 $\mu$ L reaction system containing 20 mM Tris-HCl, pH 8.0, at 37 centigrade for 2 hours.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Usage</b>           | 1. Mix 10 $\mu$ g of glycoprotein with a solution of 20 mM Tris-HCl (pH 8.0), 40 mM DTT, and 0.1% SDS to make the total volume 50 $\mu$ L. 2. Heat at 95 centigrade for 10 minutes. 3. Cool to room temperature. 4. Add 3 $\mu$ L of 500 mM iodoacetic acid solution. 5. Leave it at room temperature for 30 minutes. 6. Transfer the solution to an ultrafiltration centrifuge tube. 7. Centrifuge at 12, 000 $\times$ g for 4 minutes and discard the flow-through. 8. Add 400 $\mu$ L of 20 mM Tris-HCl (pH 8.0) solution. 9. Centrifuge at 12, 000 $\times$ g for 4 minutes and discard the flow-through. Repeat this step by adding another 400 $\mu$ L of 20 mM Tris-HCl (pH 8.0) solution. 10. Add 20 $\mu$ L of 20 mM Tris-HCl (pH 8.0) solution. 11. Add 1 $\mu$ L of O-Glycoprotease and mix gently. 12. Incubate at 37 centigrade for 2-18 hours. 13. Centrifuge at 12, 000 $\times$ g for 4 minutes and collect the supernatant. |
| <b>Applications</b>    | 1. Determination of O-glycosylation sites 2. Determination of O-glycan structures                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Note</b>            | 1. Try to avoid freeze-thaw cycles of this product after receipt; 2. Please wear lab coat and disposable gloves when using; 3. This product should not be used directly for clinical diagnosis and treatment.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Stability</b>       | This product should be stored at -20 centigrade and can be stored for at least 12 months.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Storage</b>         | Store at -20 centigrade.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Storage Buffer</b>  | 20mM Tris-HCl, 100mM NaCl, pH 7.5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Shipping</b>        | Dry Ice                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |

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