

O-Glycosidase

1 Components

Component	HY-KE7057-1 KU
O-Glycosidase	1 KU
10× Glycoprotein Denaturing Buffer	1000 µL
10× GlycoBuffer 2	1000 µL

2 Introduction

O-Glycosidase, expressed in *E.coli* and derived from *Streptococcus pneumoniae*, is highly specific and can catalyze the removal of linked disaccharides from serine, threonine residues or glycoproteins to release Gal β 1-3GalNAc. The enzyme can be applied to glycoprotein biosynthesis analysis, O-glycan bioactive protein O-glycosylation detection and binding site analysis.

3 Properties

Source	<i>E.coli</i>
Label	N-terminal His Tag
Molecular weight	180 kDa
Buffer	Supplied lyophilized. 20 mM Tris-HCl, 200 mM NaCl (pH 7.5)
Enzyme activity	\geq 10000 U/mg
Unit Definition	One unit is defined as the amount of enzyme required to catalyze the release of 1 nmol Gal β 1-3GalNAc α 1-Thr per hour at 37°C and pH 5.5.

4 General Protocol

1. Preparation

Take out the O-Glycosidase reagent, add 30-50 µL of ddH₂O to dissolve, and centrifuge at 10000 rpm for 10 s to ensure that all reagents are at the bottom of the tube. Other buffers are taken out of the -20°C refrigerator for later use.

2. Deglycosylation of glycoproteins under denaturing conditions

1) Dissolve 1-20 µg of glycoprotein in ddH₂O, add 1 µL of 10× Glycoprotein Denaturing Buffer, and adjust the volume to 10 µL with deionized water. Incubate at 75°C for 10 min.

- 2) Add 2 μL of 10 \times GlycoBuffer 2 and 2 μL of 10% NP-40, and mix by gently pipetting.
- 3) Add 1-4 μL of O-Glycosidase, add ddH₂O to 20 μL , and pipe gently to mix. Incubate at 37°C for 1-4 h.
- 4) For SDS-PAGE analysis or HPLC analysis.

3. Deglycosylation of glycoproteins under non-denaturing conditions

- 1) Add 2 μL 10 \times Glyco buffer and 2-5 μL O-Glycosidase to 10-100 μg glycoprotein solution, add ddH₂O to make the total volume of the reaction system 20 μL , and mix gently.
- 2) React at 37°C for 4-24 h.

Note: When deglycosylating natural glycoproteins, an equal amount of glycoprotein samples can be denatured as a positive control to determine the degree of deglycosylation reaction under non-denaturing conditions.

5 Storage

-20°C, 1 year

6 Precautions

1. Avoid repeated freezing and thawing.
2. This product is for R&D use only, not for drug, household, or other uses.
3. For your safety and health, please wear a lab coat and disposable gloves to operate.