

## DESCRIPTION

Formula	C <sub>17</sub> H <sub>24</sub> N <sub>6</sub> O <sub>17</sub> P <sub>2</sub>
Molecular Weight	646.35 Da
Formulation	1 mM provided in 20 mM Tris, pH 8.0
Storage & Stability	Store the unopened product at ≤ -20 °C. Good for 12 months from date of receipt.

## APPLICATIONS

- For *in vitro* enzymatic incorporation of azido-sugars into specific, targeted glycans
- Detect the presence or absence of GalNAc modifications
- Monitoring O-glycans

## KEY FEATURES & BENEFITS

- Can be introduced to proteins and lipids via various GalNAc transferases
- Can be conjugated to desired reporter molecules via click chemistry
- Can be detected via Western Blot, ELISA, and flow cytometry, depending on the type of reporter molecule
- Contain the smallest possible orthogonal functional group
- Has minimal side effect on target molecules
- User-friendly

**For Details:** [Wu et al., \(2015\) Carbohydrate Res. 412:1-6.](#)

## RELATED REAGENTS

### Click Chemistry

- [Biotinylated Alkyne](#)
- [CMP-Azido-Sialic Acid](#)
- [GDP-Azido-Fucose](#)
- [UDP-Azido-GlcNAc](#)

### Enzymes & Detection Reagents

- GalNAc specific transferases such as, [Recombinant Human GALNT1](#), [GALNT2](#), and [GALNT3](#)
- [Recombinant C. perfringens Neuraminidase](#) and [Recombinant E. faecalis O-Glycosidase](#)
- [Streptavidin-HRP](#)

## SAMPLE PROTOCOL FOR LABELING GLYCOPROTEIN WITH O-GLYCAN REPLACEMENT

Protocols are guidelines. Parameters need to be optimized by end users.

### OTHER MATERIALS REQUIRED

- Protein Sample
- Assay Buffer: 25 mM HEPES, 150 mM NaCl, 10 mM MnCl<sub>2</sub>, pH 7.5
- [Recombinant Human GALNT2 \(R&D Systems<sup>®</sup>, Catalog #7507-GT\)](#)
- [Recombinant \*C. perfringens\* Neuraminidase \(R&D Systems<sup>®</sup>, Catalog # 5080-NM\)](#)
- [Recombinant \*E. faecalis\* O-Glycosidase \(R&D Systems<sup>®</sup>, Catalog # 8886-GH\)](#)
- [Biotinylated Alkyne \(R&D Systems<sup>®</sup>, Catalog # ES100\)](#)
- CuCl<sub>2</sub>, 1 mM in deionized water
- Ascorbic Acid, 20 mM in deionized water
- SDS-PAGE and Western Blot reagents or equivalent
- TBST buffer: 25 mM Tris, 137 mM NaCl, 0.1% Tween-20, pH 7.5
- [Streptavidin-HRP \(R&D Systems<sup>®</sup>, Catalog # DY998\)](#)

### ASSAY PROCEDURE

1. Prepare a reaction mixture by combining 5.0 µg Protein Sample, with 1.0 µg Recombinant Human GALNT2, 0.2 µg Recombinant *E. faecalis* O-Glycosidase, 0.1 µg Recombinant *C. perfringens* Neuraminidase, 0.5 nmol UDP-Azido-GalNAc in the Assay Buffer with the final volume of 25 µL.
2. Prepare negative controls according to step 1 but omit Recombinant Human GALNT2 or UDP-Azido-GalNAc.
3. Incubate reactions and controls at 37 °C for 30 minutes.
4. To each of the samples, add 5.0 µL of 1 mM CuCl<sub>2</sub>, 5.0 µL of 20 mM Ascorbic Acid, and 5.0 µL of 1 mM Biotinylated Alkyne. Mix with gentle tapping.
5. Incubate all samples at room temperature for 1 hour.
6. Separate the reactions and controls by SDS-PAGE.
7. Blot the gel to a nitrocellulose membrane.
8. Block the blot with 10% fat-free milk for 5 minutes.
9. Thoroughly wash the membrane with TBST buffer by changing buffer three times for a total of 45 minutes.
10. Incubate the blot with 25 ng/mL Streptavidin-HRP in 30 mL TBST buffer for 30 minutes.
11. Thoroughly wash the membrane with TBST buffer by changing buffer three times for a total of 45 minutes.
12. Detect with commercial ECL (Enhanced Chemiluminescence) reagents.

### FINAL ASSAY CONDITIONS PER REACTION

- UDP-Azido-GalNAc: 0.5 nmol
- Recombinant Human GALNT2: 1.0 µg
- Recombinant *C. perfringens* Neuraminidase: 0.1 µg
- Recombinant *E. faecalis* O-Glycosidase : 0.2 µg
- Protein Sample: 5.0 µg
- Reaction volume: 25 µL

### CLICK CHEMISTRY REACTION CONDITIONS PER REACTION

- CuCl<sub>2</sub>: 5 nmol
- Ascorbic Acid: 100 nmol
- Biotinylated Alkyne: 5 nmol
- Reaction volume: 40 µL