R&D SYSTEMS
a bio-techne brand

UDP-Azido-GlcNAc

Catalog Number: ES104-100
Lot Number: DKFA02
Size: 100 µg

DESCRIPTION

Formula	$C_{17}H_{24}N_6O_{17}P_2$
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.
- Detecting the presence or absence of terminal GlcNAc residues (including O-GlcNAc).
- Detecting T and Tn antigens.
- Detecting the presence of high mannose glycan.

KEY FEATURES AND BENEFITS

- Can be introduced to proteins and lipids via various GlcNAc 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.
- Contains the smallest possible orthogonal functional group.
- Has minimal side effects 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
- UDP-Azido-GalNAc
- GDP-Azido-Fucose

Enzymes & Detection Reagents

- GlcNAc specific transferases such as, Recombinant Human MGAT1 , B3GNT6 , GCNT1 and OGT
- Hexaminodases such as, Recombinant Human HEXA and HEXB , Recombinant *B. thetaiotaomicron* OGA
- Streptavidin-HRP

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SAMPLE PROTOCOL FOR GLcNAc DETECTION ON CORE-1 O-GLYCAN

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

OTHER MATERIALS REQUIRED

- Assay Buffer: 25 mM Tris, 150 mM NaCl, 10 mM $MnCl_2$, pH 7.5
- Protein Sample
- Recombinant Human GCNT1 (R&D Systems®, Catalog # 7248-GT)
- Biotinylated Alkyne (R&D Systems, Catalog # ES100)
- $CuCl_2$, 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, Catalog # DY998)

FINAL ASSAY CONDITIONS PER REACTION

- UDP-Azido-GlcNAc: 1 nmol
- Recombinant Human GCNT1: 1 µg
- Protein Sample: 5 µg
- Reaction volume: 25 µL

CLICK CHEMISTRY REACTION CONDITIONS PER REACTION

- $CuCl_2$: 5 nmol
- Ascorbic Acid: 100 nmol
- Biotinylated Alkyne: 5 nmol
- Reaction volume: 40 µL

ASSAY PROCEDURE

1. Prepare a reaction mixture by combining 5.0 µg of Protein Sample, 1 µg of Recombinant Human GCNT1 in the presence of 1 nmol of UDP-Azido-GlcNAc in the Assay Buffer with the final volume of 25 µL.
2. Prepare negative controls according to step 1 but omit Protein Sample or Recombinant Human GCNT1.
3. Incubate all the reactions and the controls at 37 °C for one hour.
4. To each of the samples, add 5 µL of 1 mM $CuCl_2$, 5.0 µL of 20 mM Ascorbic Acid, and 5 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.