

DESCRIPTION

Formula	C ₂₀ H ₃₀ N ₇ O ₁₅ P
Molecular Weight	639.46 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 sialated glycans.
- Labeling terminal sialoglycans on proteins and lipids.

KEY FEATURES & BENEFITS

- Can detect terminal sialoglycans without specialized, expensive equipment.
- Can be introduced to proteins and lipids via various sialyltransferases.
- Can be conjugated to desired reporter molecules, such as biotin, via click chemistry.
- Can be detected via Western Blot, ELISA, and flow cytometry, depending on the type of reporter molecule.
- 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](#)
- [GDP-Azido-Fucose](#)
- [UDP-Azido-GalNAc](#)
- [UDP-Azido-GlcNAc](#)

Enzymes and Detection Reagents

- [Sialyltransferases and related enzymes](#)
- [Neuraminidases/Sialidases and related enzymes](#)
- [Streptavidin-HRP](#)

SAMPLE PROTOCOL FOR LABELING & DETECTING O-GLYCAN ON FETUIN WITH ST3GAL1

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

OTHER MATERIALS REQUIRED

- Assay Buffer: 25 mM HEPES, 150 mM NaCl, 10 mM MnCl₂, pH 7.5
- Fetuin (Sigma), 50 mg/mL stock in deionized water
- [Recombinant Human ST3GAL1 \(R&D Systems®, Catalog #6905-GT\)](#)
- [Recombinant C. perfringens Neuraminidase \(R&D Systems®, Catalog # 5080-NM\)](#)
- [Biotinylated Alkyne \(R&D Systems®, Catalog # ES100\)](#)
- MnCl₂, 100 mM in deionized water
- CuCl₂, 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 \(R&D Systems®, Catalog # DY998\)](#)

ASSAY PROCEDURE

1. Prepare Desialylated Fetuin by mixing Fetuin with *rcp*Neuraminidase at mass ratio of 100:1 in the Assay Buffer with the concentration of Fetuin at 1.0 mg/mL for 20 minutes at room temperature. The *rcp*Neuraminidase is then heat inactivated at 90 °C for 5 minutes.
2. Prepare reaction mixture by combining 5.0 µg Desialylated Fetuin, 1.0 µg Recombinant Human ST3GAL1, 0.5 nmol CMP-Azido-Sialic Acid, in the Assay Buffer supplemented with 10 mM MnCl₂ with the final volume of 25 µL.
3. Prepare negative controls according to step 2 but omit Recombinant Human ST3GAL1 or CMP-Azido-Sialic Acid.
4. Incubate reactions and controls at 37 °C for 30 minutes.
5. To each of the samples, add 5.0 µL of 1 mM CuCl₂, 5.0 µL of 20 mM Ascorbic Acid, and 5.0 µL of 1 mM Biotinylated Alkyne. Mix with gentle tapping. 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

- CMP-Azido-Sialic Acid: 0.5 nmol
- rhST3GAL1: 1.0 µg
- Desialylated Fetuin: 5.0 µg
- Reaction volume: 25 µL

CLICK CHEMISTRY REACTION CONDITIONS PER REACTION

- CuCl₂: 5 nmol
- Ascorbic Acid: 100 nmol
- Biotinylated Alkyne: 5 nmol
- Reaction volume: 40 µL