

Description

Formula	$C_{16}H_{24}N_8O_{15}P_2$
Molecular Weight	630.35 Da
Formulation	Provided in 20 mM Tris, 50 mM NaBorate, pH 9.0
Storage & Stability	Store the unopened product at < -20 °C. Good for 12 months from date of receipt.

Incorporate or detect fucose without expensive, specialized equipment!

Applications

- Detecting the presence or absence of fucosylated glycans.
- Tagging bio-molecules through fucosylation.
- Assessing the level of fucosylation on target molecules.

Key Features and Benefits

- Can assess for the presence of terminal fucosylation (e.g. core fucose) without expensive, specialized equipment.
- Can be introduced to proteins and lipids that have fucosylation sites via fucosyltransferases.
- 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](#)
- [UDP-Azido-GlcNAc](#)

Enzymes and Detection Reagents

- Fucosyltransferases such as [Recombinant Human FUT3](#), [FUT5](#), [FUT6](#), [FUT7](#) and [FUT8](#)
- [Recombinant Human Tissue alpha-L-Fucosidase](#) and [Recombinant T. maritima alpha-L-Fucosidase](#)
- [Streptavidin-HRP](#)

Sample Protocol for Core-6 Fucose Labeling

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

Materials

- Assay Buffer: 25 mM HEPES, 150 mM NaCl, 10 mM MnCl₂, pH 7.5.
- Protein Sample
- [Recombinant Human Fucosyltransferase 8/FUT8 \(R&D Systems, Catalog # 5768-GT\)](#)
- GDP-Azido-Fucose (R&D Systems, Catalog # ES101)
- [Biotinylated Alkyne \(R&D Systems, Catalog # ES100\)](#)
- 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** a reaction mixture by combining 5 µg of Protein Sample, 1 µg of rhFUT8 in the presence of 1 nmol of GDP-Azido-Fucose in the Assay Buffer with the final volume of 25 µL.
- 2. Prepare** negative controls according to step 1 but omit Protein Sample or rhFUT8 or GDP-Azido-Fucose.
- 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 CuCl₂, 5 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 12% 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

- GDP-Azido-Fucose: 1 nmol
- rhFUT8: 1 µg
- Protein Sample: 5 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