

DESCRIPTION

Formula	C ₄₁ H ₆₅ N ₁₀ O ₂₁ PS
Molecular Weight	1097.05 Da
Formulation	Lyophilized with Tris, at pH 8.0
Storage & Stability	Store the unopened product at ≤ -20 °C. Good for 12 months from date of receipt.

APPLICATIONS

- Allow direct biotinylation of sialoglycans of proteins and lipids.
- Detecting specific sialoglycans on glycoproteins.
- Detecting and imaging sialoglycans on cells and tissue samples.

KEY FEATURES & BENEFITS

- Can be introduced to sialoglycans and glycolipids via various sialyltransferases.
- Allows glycan imaging on live cells.
- No side effects on target molecules observed.
- Convenient and user-friendly.

For Details:

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

[Wu et al. \(2019\) Glycobiology, 29:750-754.](#)

RELATED REAGENTS

Click Chemistry

- [Biotinylated Alkyne \(ES100\)](#)
- [GDP-Azido-Fucose \(ES101\)](#)
- [CMP-Azido-Sialic Acid \(ES102\)](#)
- [UDP-Azido-GalNAc \(ES103\)](#)
- [UDP-Azido-GlcNAc \(ES104\)](#)
- [GDP-Cy5-Fucose \(ES301\)](#)
- [CMP-Cy5-Siaic Acid \(ES302\)](#)
- [GDP-Cy3-Fucose \(ES401\)](#)
- [CMP-Cy3-Sialic Acid \(ES402\)](#)

Enzymes and Detection Reagents

- [Various sialyltransferases](#)
- [Various neuraminidases/sialidases](#)
- [Streptavidin-HRP \(DY998\)](#)

SAMPLE PROTOCOL FOR LABELING & DETECTING SIALOGLYCANS WITH BIOTINYLATED SIALIC ACID

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

OTHER MATERIALS REQUIRED

- Assay Buffer: 25 mM Tris, 10 mM MnCl₂, pH 7.5
- Sample glycoprotein
- [Recombinant sialyltransferases](#)
- [Recombinant neuraminidase](#)
- 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 \(R&D Systems®, Catalog # DY998\)](#)
- 10% fat free milk

FINAL ASSAY CONDITIONS PER REACTION

- CMP-C9-Biotin-Sialic Acid (R&D Systems®, Catalog # ES201): 1 nmol
- Recombinant sialyltransferase: 0.5 µg
- Recombinant neuraminidase: 0.1 µg
- Sample glycoproteins: 1-10 µg

Table 1. Enzyme selection for Glycan Labeling

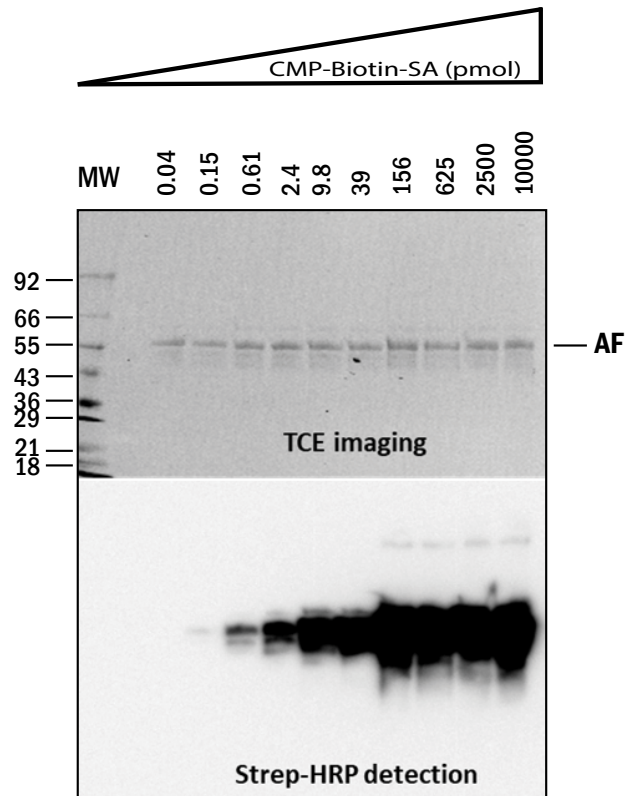
GLYCANS TO BE LABELED	LABELING ENZYMES (R&D SYSTEMS, CATALOG #)	NEURAMINIDASE (R&D SYSTEMS, CATALOG #)
N-Glycan	ST6GAL1 (7620-GT) ST3GAL4 (10496-GT) ST3GAL6 (10591-GT)	C. perfringens Neuraminidase (5080-NM)
O-Glycan	ST3GAL1 (6905-GT) ST3GAL2 (7275-GT) ST6GAINAC1 (9154-GT) ST6GAINAC2 (6468-GT) ST6GAINAC3 ST6GAINAC4 (6876-GT)	C. perfringens Neuraminidase (5080-NM)
Polysialic acid (PSA)	ST8SIA1 (6716-GT) ST8SIA2 (6590-GT) ST8SIA4 (7027-GT) ST8SIA6 (9587-GT)	M. viridifaciens Neuraminidase (5084-NM)

ASSAY PROCEDURE

1. Prepare reaction mixture by combining a sample glycoprotein (ideally between 1-10 µg), 0.5 µg of a recombinant sialyltransferase, 0.1 µg of recombinant neuraminidase, 1 nmol CMP-C9-Biotin-Sialic Acid, in the Assay Buffer with the final volume of 25 µL.
2. Prepare negative controls by combining a sample glycoprotein (ideally between 1-10 µg), 0.1 µg of recombinant neuraminidase, 1 nmol CMP-C9-Biotin-Sialic Acid in the Assay Buffer with the final volume of 25 µL.
3. Incubate reactions and controls at 37 °C for 30 minutes.
4. Separate the reactions and controls by SDS-PAGE.
5. Blot the gel to a nitrocellulose membrane.
6. Block the blot with 10% fat-free milk for 5 minutes.
7. Thoroughly wash the membrane with TBST buffer by changing buffer three times for a total of 30 minutes.
8. Incubate the blot with 25 ng/mL Streptavidin-HRP in 30 mL TBST buffer for 30 minutes.
9. Thoroughly wash the membrane with TBST buffer by changing buffer three times for a total of 45 minutes.
10. Detect with commercial ECL (Enhanced Chemiluminescence) reagents.

SAMPLE DATA

Titration of CMP-C9-Biotin-Sialic Acid on Asialofetuin



Labeling and detection of N-glycans on fetal bovine fetuin. Asialofetuin (AF) was prepared from fetal bovine fetuin by treatment with Recombinant *C. perfringens* Neuraminidase, CF ([Catalog # 5080-NM](#)) and purified through size exclusion chromatography. Asialofetuin (8 μg) was incubated with indicated amounts of CMP-C9-Biotin-Sialic Acid ([Catalog # ES201](#)) with a constant amount of Recombinant Human ST6GAL1 (aa 44-406) Protein ([Catalog # 7620-GT](#)) (0.1 μg) in 25 μL of 25 mM Tris, 10 mM MnCl_2 , pH 7.5 buffer at 37 $^\circ\text{C}$ for 30 minutes. The reactions were first separated on an SDS-PAGE and imaged by trichloroethylene (TCE) staining (upper panel). The separated proteins were then blotted to a nitrocellulose membrane and detected with conventional Streptavidin-HRP ([Catalog # DY998](#)) chemiluminescence reagents (lower panel). It is concluded that 0.1 nmol of CMP-C9-Biotin-Sialic Acid will achieve saturating labeling.