

## DESCRIPTION

<b>Formula</b>	$C_{52}H_{73}N_{10}O_{25}P_1S_3$
<b>Molecular Weight</b>	1413.25 Da
<b>Formulation</b>	Lyophilized with Tris, at pH 8.0
<b>Storage &amp; Stability</b>	Store the unopened product at $\leq -20$ °C. Good for 12 months from date of receipt.

## APPLICATIONS

- Fluorescent labeling with Cy3 of free glycans as well as glycoproteins and glycolipids.
- Fluorescent detection of specific glycan epitopes on the cell surface.
- Quantitation of the sialylation level of specific glycans.
- Together with CMP-Cy5-Sialic Acid, allows for dual labeling and detection of sialoglycans.

## KEY FEATURES & BENEFITS

- Excitation at 550 nm and emission at 570 nm, exhibits green fluorescent light under microscope.
- The fluorescent dye Cy3 is conjugated to the C9 position of the sialic acid.
- Can be directly introduced into glycoproteins and glycolipids via various sialyltransferases.
- Can be introduced to live cells for glycan imaging.
- Have minimum side-effect on target molecules.
- Very convenient and user-friendly.

## RELATED REAGENTS

### Click Chemistry

- [Biotinylated-Alkyne \(ES100\)](#)
- [GDP-Azido-Fucose \(ES101\)](#)
- [CMP-Azido-Sialic Acid \(ES102\)](#)
- [UDP-Azido-GalNAc \(ES103\)](#)
- [UDP-Azido-GlcNAc \(ES104\)](#)
- [CMP-C9-Biotin-Sialic Acid \(ES201\)](#)
- GDP-Cy5-Fucose (ES301)
- [CMP-Cy5-Sialic Acid \(ES302\)](#)
- GDP-Cy3-Fucose (ES401)

### Enzymes and Detection Reagents

- [Various sialyltransferases](#)
- [Various neuraminidases/sialidases](#)

## SAMPLE PROTOCOL FOR DIRECT FLUORESCENT GLYCAN LABELING WITH CMP-CY3-SIALIC ACID

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

### OTHER MATERIALS REQUIRED

- Sample protein
- Assay Buffer: 25 mM Tris, 10 mM MnCl<sub>2</sub>, pH 7.5
- Sialyltransferases such as rhST3GAL1 ([R&D Systems®](#), [Catalog # 6905-GT](#)) or rhST6GAL1 ([R&D Systems](#), [Catalog # 7620-GT](#))
- Recombinant C. perfringens Neuraminidase ([R&D Systems®](#), [Catalog# 5080-NM](#))
- Protein sample loading dye
- SDS-PAGE and Western Blot reagents or equivalent
- Fluorescent Imager in a green fluorescent channel

### FINAL ASSAY CONDITIONS PER REACTION

- Sample protein: 0.1 to 5 µg
- CMP-Cy3-Sialic Acid: 0.2 nmol
- Sialyltransferase: 0.5 µg
- Neuraminidase: 0.1 µg

### ASSAY PROCEDURE

1. Prepare a reaction mixture by combining 0.1-5 µg of a sample protein, 0.2 nmol CMP-Cy3-Sialic Acid, 0.5 µg of a sialyltransferase such as ST3GAL1 or ST6GAL1, 0.1 µg of rcpNeuraminidase, add Assay Buffer to the final volume to 30 µL.
2. Prepare a negative control by repeating above but omitting the sialyltransferase.
3. Incubate all the reactions and controls at 37 °C for 60 minutes.
4. Stop the reactions and controls by adding appropriate volume of protein sample loading dye to each reaction.
5. Separate the reactions and controls by SDS-PAGE.
6. Image the gel with a fluorescent imager in a green fluorescent channel.
7. Image the gel with trichloroethanol (TCE) imaging (if TCE is incorporated into the gel) or any other regular protein gel imaging method such as Coomassie® blue staining or silver staining.