R&D SYSTEMS
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CMP-Cy5-Sialic Acid

Catalog Number: ES302-050
Lot Number: DNOK01
Size: 50 µg

DESCRIPTION

Formula	$C_{57}H_{75}N_{10}O_{25}P_1S_3$
Molecular Weight	1427.42 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

- Fluorescent labeling with Cy5 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-Cy3-Sialic Acid, allows for dual labeling and detection of sialoglycans.

KEY FEATURES & BENEFITS

- Excitation at 649 nm and red emission at 671 nm.
- The fluorescent dye Cy5 is conjugated to the C9 position of sialic acid.
- Can be directly introduced into glycoproteins and glycolipids via various sialyltransferases.
- Can be introduced to live cells for glycan imaging.
- Has minimum side-effects 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-Biotin-Sialic Acid (ES201)

Enzymes and Detection Reagents

- Various sialyltransferases
- Various neuraminidases/sialidases

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SAMPLE PROTOCOL FOR DIRECT FLUORESCENT GLYCAN LABELING WITH CMP-CY5-SIALIC ACID

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

OTHER MATERIALS REQUIRED

- Assay Buffer: 25 mM Tris, 10 mM $MnCl_2$, pH 7.5
- Sample protein
- Recombinant 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 far-red fluorescent channel

FINAL ASSAY CONDITIONS PER REACTION

- Sample protein: 0.1 to 5 µg
- CMP-Cy5-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-Cy5-Sialic Acid, 0.5 µg of a sialyltransferase such as ST3GAL1 or ST6GAL1, 0.1 µg of rCpNeuraminidase in the final Assay Buffer with the final volume to 30 µL.
2. Prepare a negative control by repeating above but omitting the sialyltranferase.
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 far-red 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.