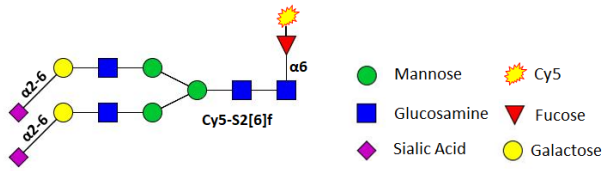
Catalog Number	Size
GL501	100 pmol

Cy5-Fuc Labeled S2[6]f (Cy5-S2[6]f)



Description

Formula	Fuc1Neu5Ac2Gal2Man3GlcNAc4Cy5
Predicted Mass	3197.63 Da
Formulation	Supplied in 20 mM Tris, pH 8.0
Storage & Stability	• 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening. • Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

Applications

- Used as a ligand for lectins and to study glycan protein interaction.
- Used as a substrate for various glycosidases and glycosyltransferases.
- Used as a substrate neuraminidases activity and specificity studies.

Key Features & Benefits

- Excitation at 649 nm and emission at 671 nm.
- The fluorescent dye Cy5 is conjugated to the C6 position of the core-6 fucose.
- Can be separated on 15-17% SDS-PAGE and directly visualized as a single band through the red channel of a fluorescent imager.
- Linear response range for Cy5-labeled glycans can be from 10 fmol to 100 pmol, depending on the sensitivity of detection.

Related Reagents

CLICK CHEMISTRY

- GDP-Cy5-Fucose (ES301)
- CMP-Cy5-Sialic Acid (ES302)
- GDP-Cy3-Fucose (ES401)
- CMP-Cy3-Sialic Acid (ES402)

LABELED GLYCANS

- Cy5-Fuc Labeled M1N1f (GL301)
- Cy5-Fuc Labeled Glycan G2f (GL302)
- Cy5-Fuc Labeled N2f (GL304)
- Fluorescent Glycan Labeling and Detection

ENZYMES AND DETECTION REAGENTS

- Various sialytransferases
- Various fucosyltransferases
- Various neuraminidases/sialidases

Sample Assay Protocol

Testing Activity of Neuraminidases using Cy5-Fuc Labeled S2[6]f/Cy5-S2[6]f as a substrate.

Suggested input of Cy5-S2[6]f in an assay separated on SDS-PAGE is from 0.01-1 pmol.

Protocols are guidelines. Parameters need to be optimized by end users, enzyme input and reaction time may need to be adjusted to accommodate specific activity of the enzyme.

Refer to Wu, ZL. et al. (2020) Glycobiology, 30:970. https://academic.oup.com/glycob/article/30/12/970/5815178

Other Materials Required

- Assay Buffer: 50 mM NaOAc, pH 4.5 (dependent on requirements of Neuraminidases)
- Neuraminidases
- 15% SDS-PAGE
- 6X Gel Loading Dye
- Fluorescent imager

Final Assay Conditions Per Reaction

- Neuraminidases: 1-10 µg
- Cy5-S2[6]f: 0.2 pmol

Assay Procedure

1. Dilute Neuraminidase 10-100 ng/µL in the Assay Buffer.
2. Dilute Cy5-S2[6]f to 0.02 µM in Assay Buffer.
3. Mix 10 µL dilute Neuraminidase and 10 µL of Cy5-S2[6]f in a centrifuge tube.
4. Prepare a negative control by mixing 10 µL of Cy5-S2[6]f with 10 µL of Assay Buffer.
5. Incubate the reaction and control at 37 °C for 1 hour.
6. Stop the reactions and controls by adding 4 µL of 6X Gel Loading Dye to each tube.
7. Load 12 µL of each of the above reactions and controls per well on a 15% SDS-PAGE and run down 80% length of the gel.
8. Image the gel using a fluorescent imager using the red channel for 10 seconds.