# GDP-Cy5-Fucose

**Catalog Number:** ES301-10
**Lot Number:** DLHD01
**Size:** 10 µg

## DESCRIPTION

| | |
|---|---|
| **Formula** | $C_{53}H_{67}N_{11}O_{25}P_2S_3$ |
| **Molecular Weight** | 1416.3 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 glycoproteins as well as cell surface.
- Quantitation of the fucosylation 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 emission at 671 nm, exhibits red fluorescent light under microscope.
- The fluorescent dye is conjugated to the C6 position of the fucose.
- Can be directly introduced into glycoproteins and glycolipids via various fucosyltransferases.
- Can be introduced to live cells for glycan imaging.
- Has minimum side-effects on target molecules.
- Very convenient and user-friendly.

**For Details:**
Wu, ZL. *et al.*, (2020) Glycobiology **30**:970.

## RELATED REAGENTS

### Click Chemistry

- CMP-Cy5-Sialic Acid (ES302)
- 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 fucosyltransferases
- Various neuraminidase
- Various fucosidase



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## SAMPLE PROTOCOL

**For direct fluorescent glycan labeling with GDP-Cy5-Fucose. 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 fucosyltransferases such as rhFUT9 (R&D Systems®, Catalog # 9347-GT) or rhFUT8 (R&D Systems®, Catalog # 5768-GT)
- 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
- GDP-Cy5-Fucose: 0.2 nmol
- Fucosyltransferase: 0.5 µg

## ASSAY PROCEDURE

1. Prepare a reaction mixture by combining 0.1 to 5 µg of a sample protein, 0.2 nmol GDP-Cy5-Fucose, 0.5 µg of a fucosyltransferases such as FUT9 or FUT8, add Assay Buffer to the final volume to 30 µL.
2. Prepare a negative control by repeating above but omitting the fucosyltransferases.
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.

**Notes:**

- FUT9 does not recognize sialylated lactosamine. To increase FUT9 labeling on samples that are highly sialylated, small amount of Recombinant C. perfringens Neuraminidase Protein, CF (R&D Systems®, Catalog # 5080-NM) may by added into the labeling reaction mixture to increase labeling.
- Recombinant Human Tissue alpha-L-Fucosidase/FUCA1, CF (R&D Systems®, Catalog #7039-GH) may by used to remove existing fucose residue on samples prior to the reaction to increase labeling. Since the fucosidase is active around pH 4.5 and requires $Mg^{2+}$, buffer should be conditioned to that of fucosyltransferases after the treatment of fucosidase.