

Background

Because N-, O-glycans and glycolipids are frequently fucosylated at terminal sites, fucose is often found to be essential for sugar epitope and lectin ligand generation. Well known fucose containing structures include Lewis structures and ABO blood group antigens. Lewis structures are key elements involved in leukocyte homing and extravasation process and thus are essential for lymphocyte maturation and natural defense functions. Fucose containing glycans also play essential roles in cell signaling and development. So far, more than 10 Fucosyltransferases have been cloned from the human genome (1). FUT1 and FUT2 are α -2 Fucosyltransferases and are responsible for ABO blood group antigen synthesis. FUT3, FUT4, FUT5, FUT6, FUT7 and FUT9 are α -1-3/4 Fucosyltransferases and are responsible for Lewis structure generation. FUT5 has high homology with FUT3 and FUT6 due to gene duplication. FUT7 is exclusively responsible for biosynthesis of sialyl Lewis X epitope in leukocytes and high endothelial venule cells (2). FUT8 is an α -6 Fucosyltransferase that adds a fucose to the chitobiose core of N-glycans (3). Predicted as type II transmembrane proteins and Golgi enzymes, some of the Fucosyltransferases can also be found in plasma. R&D Systems rhFUTs correspond to the luminal domains. The activity of this enzyme has been measured using a phosphatase-coupled method (4).

References:

1. Becker, D.J. *et al.* (2003) *Glycobiology* **13**:41R.
2. Blander, J. M. *et al.* (1999) *J. Immunol.* **163**:3746.
3. Lee, S.H. *et al.* (2006) *J. Biochem.* **139**:391.
4. Wu, Z.L. *et al.* (2010) *Glycobiology* doi: 10.1093/glycob/cwq187.

Description

Source	Murine myeloma cell line, NS0-derived Arg35 - Thr374, with an N-terminal 6-His tag Accession # Q11128
N-terminal Sequence Analysis	His
Structure / Form	Disulfide-linked homodimer
Predicted Molecular Mass	40 kDa

Specifications

SDS-PAGE	58 kDa, reducing conditions
Activity	Measured by its ability to transfer fucose from GDP-fucose to <i>N</i> -Acetyllactosamine The specific activity is > 35 pmol/min/ μ g, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 μ g of the protein by the LAL method.
Purity	>80%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 μ m filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Preparation and Storage

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 6 months from date of receipt, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 5 mM MnCl₂ (supplied in kit), pH 7.5
- Recombinant Human Fucosyltransferase 5/FUT5 (rhFUT5) (R&D Systems, Catalog # 4949-GT)
- Donor Substrate: GDP-Fucose (Sigma, Catalog # G4401), 1.6 mM stock in deionized water
- Acceptor Substrate: Lactosamine (V-Labs, Catalog # GN204), 50 mM in deionized water
- Glycosyltransferase Activity Kit (R&D Systems, Catalog # EA001)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute GDP-Fucose to 240 μ M in Assay Buffer.
2. Dilute Lactosamine to 2.4 mM in Assay Buffer.
3. Dilute Coupling Phosphatase 1 to 12 μ g/mL in Assay Buffer.
4. Prepare reaction mixture by combining equal volumes of diluted GDP-Fucose, Lactosamine, and Coupling Phosphatase 1.
5. Dilute rhFUT5 to 20 μ g/mL in Assay Buffer.
6. Dilute 1 mM Phosphate Standard by adding 40 μ L of the 1 mM Phosphate Standard to 360 μ L of Assay Buffer for a 100 μ M stock.
7. Prepare standard curve by performing seven one-half serial dilutions of the 100 μ M Phosphate stock in Assay Buffer. The standard curve has a range of 0.039 to 2.5 nmoles per well.
8. Load 50 μ L of each dilution of the standard curve into a plate. Include a curve blank containing 50 μ L of Assay Buffer.
9. Load 25 μ L of the 20 μ g/mL rhFUT5 into the plate. Include a Blank Control containing 25 μ L of Assay Buffer.
10. Add 25 μ L of reaction mixture (step 4) to the wells, excluding the standard curve and curve blank.
11. Cover the plate with parafilm or a plate sealer and incubate at 37 °C for 20 minutes.
12. Add 30 μ L of the Malachite Green Reagent A to all wells. Tap to mix briefly.
13. Add 100 μ L of deionized water to all wells. Tap to mix briefly.
14. Add 30 μ L of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
15. Read plate at 620 nm (absorbance) in endpoint mode.
16. Calculate specific activity:

$$\text{Specific Activity (pmol/min}/\mu\text{g)} = \frac{\text{Phosphate released}^* \text{ (nmol)} \times (1000 \text{ pmol/nmol})}{\text{Incubation time (min)} \times \text{amount of enzyme } (\mu\text{g})}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Blank Control.

Final Assay Conditions Per Reaction

- rhFUT5: 0.5 μ g
- Coupling Phosphatase 1: 0.1 μ g
- Lactosamine: 400 μ M
- GDP-Fucose: 40 μ M

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