

## 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  $\alpha$ -2 Fucosyltransferases and are responsible for ABO blood group antigen synthesis. FUT3, FUT4, FUT5, FUT6, FUT7 and FUT9 are  $\alpha$ -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  $\alpha$ -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.

## 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.

## Description

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

## Specifications

<b>SDS-PAGE</b>	58 kDa, reducing conditions
<b>Activity</b>	Measured by its ability to transfer fucose from GDP-fucose to <i>N</i> -Acetyllactosamine The specific activity is > 10 pmoles/min/ $\mu$ g, as measured under the described conditions.
<b>Endotoxin Level</b>	<1.0 EU per 1 $\mu$ g of the protein by the LAL method.
<b>Purity</b>	>80%, by SDS-PAGE under reducing conditions and visualized by silver stain.
<b>Formulation</b>	Supplied as a 0.2 $\mu$ m filtered solution in Tris and NaCl. See Certificate of Analysis for details.

## Preparation and Storage

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

## Activity Assay Protocol

### Materials

- Assay Buffer: 20 mM Tris, 20 mM MnCl<sub>2</sub>, pH 7.0
- Washing Buffer: 5 mM Tris, pH 7.0
- Recombinant human Fucosyltransferase-3 (rhFUT5) (R&D Systems, Catalog # 4949-GT)
- Donors, GDP-fucose (Sigma, Catalog # Sigma G4401) 1.6 mM stock in deionized water, GDP-<sup>3</sup>H-fucose (Perkin-Elmer, Catalog # NET989050UC) supplied at 6  $\mu$ M
- Acceptor, *N*-acetyllactosamine (V-labs, Catalog # GN204) 50 mM stock in deionized water
- Ethanol (Aldrich, Catalog # 652261)
- Bio-spin column (Biorad, Catalog # 732-6008)
- DEAE fast flow sepharose (Pharmacia, Catalog # 17-0709-01)
- Liquid Scintillation Counter (Beckman, Model # LS5000TD) or equivalent
- Liquid Scintillation Fluid (Research Products International, Catalog # 111175)

### Assay

1. Prepare 12 columns by packing with 0.4 mL of resuspended DEAE fast flow media (ensure bed volume is  $\geq$  0.5 cm).
2. Wash columns with 1 mL of diH<sub>2</sub>O.
3. Equilibrate columns with 1 mL Washing Buffer.
4. Prepare enzyme by diluting rhFUT5 to 1.0  $\mu$ g/mL in Assay Buffer.
5. Dilute substrate donors, GDP-fucose and GDP-<sup>3</sup>H-fucose to 65  $\mu$ M and to ~ 1  $\mu$ M, respectively, in Assay Buffer.
6. Prepare donor mixture by combining 128  $\mu$ L GDP-fucose and 32  $\mu$ L GDP-<sup>3</sup>H-fucose. Sufficient for ~13 reactions.
7. Combine 15  $\mu$ L rhFUT5 with 5  $\mu$ L *N*-acetyllactosamine and start reaction by adding 10  $\mu$ L of donor mixture (prepare three replicates). Include acceptor-less controls by combining 15  $\mu$ L rhFUT-3 with 5  $\mu$ L Assay Buffer and 10  $\mu$ L donor mixture.
8. Incubate at room temperature for 20 minutes.
9. Add 30  $\mu$ L of ethanol to stop each reaction. Mix well.
10. Apply 20  $\mu$ L of each reaction to a column. Elute into scintillation vial.
11. Wash each column with 1 mL Washing Buffer, elute into same scintillation vial.
12. With remaining donor mixture combine 20  $\mu$ L assay buffer, 10  $\mu$ L donor mixture and 30  $\mu$ L ethanol. Transfer 20  $\mu$ L of this mixture directly into a scintillation vial along with 1.0 mL Washing Buffer (for the determination of total applied radioisotope counts).
13. Add 5 mL liquid scintillation solution to all vials and count using liquid scintillation counter.
14. Enzyme Activity calculation:

$$\text{Specific Activity (pmol/min}/\mu\text{g)} = \frac{S \text{ (pmoles)} \times \text{Ci (incorporated counts)*}}{\text{Ct (total counts)} \times \text{time (min)} \times \text{amount of enzyme } (\mu\text{g})}$$

\*Adjusted for acceptor-less controls

### Final Assay Conditions Per Reaction

- Total applied radioisotope (S): 520 pmoles (excludes contribution from GDP-<sup>3</sup>H-fucose)
- rhFUT5: 0.015  $\mu$ g

9/30/2009

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