Recombinant Human Fucosyltransferase 3/FUT3

Catalog Number: 4950-GT

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Arg35-Thr361, with an N-terminal 6-His tag Accession # P21217
N-terminal Sequence Analysis	His
Predicted Molecular Mass	39 kDa

SPECIFICATIONS

SDS-PAGE	41 kDa, reducing conditions
Activity	Measured by its ability to transfer fucose from GDP-fucose to *N*-Acetyllactosamine The specific activity is >25 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 5 mM $MnCl_2$(supplied in kit), pH 7.5
- Recombinant Human Fucosyltransferase 3/FUT3 (rhFUT3) (Catalog # 4950-GT)
- GDP-Fucose (Sigma, Catalog # G4401), 1.6 mM stock in deionized water
- Lactosamine (V-Labs, Catalog # GN204), 50 mM in deionized water
- Glycosyltransferase Activity Kit (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 µM in Assay Buffer.
2. Dilute Lactosamine to 2.4 mM in Assay Buffer.
3. Dilute Coupling Phosphatase 1 to 12 µg/mL in Assay Buffer.
4. Prepare reaction mixture by combining equal volumes of diluted GDP-Fucose, Lactosamine, and Coupling Phosphatase 1.
5. Dilute rhFUT3 to 40 µg/mL in Assay Buffer.
6. Dilute Phosphate Standard by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of deionized water for a 100 µM stock.
7. Prepare standard curve by performing seven one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer. The standard curve has a range of 0.039 to 2.5 nmol per well.
8. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
9. Load 25 µL of the 40 µg/mL rhFUT3 into the plate. Include a Substrate Blank containing 25 µL of Assay Buffer.
10. Add 25 µL 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 µL of the Malachite Green Reagent A to all wells. Mix briefly.
13. Add 100 µL of deionized water to all wells. Mix briefly.
14. Add 30 µL 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:

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

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

Final Assay Conditions

Per Reaction:

- rhFUT3: 1 µg
- Coupling Phosphatase 1: 0.1 µg
- Lactosamine: 400 µM
- GDP-Fucose: 40 µM

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.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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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 α1-2 fucosyltransferases and are responsible for ABO blood group antigen synthesis. FUT3, FUT4, FUT5, FUT6, FUT7 and FUT9 are responsible for Lewis structure generation through their α1-3 or α1-4 fucosyltransferases activities. FUT3, also known as Lewis blood group fucosyltransferase, is unique by having both strong α1-3 and α1-4 fucosyltransferase activities (2). FUT3 has high homology with FUT5 and FUT6 due to gene duplication. FUT7 is exclusively responsible for biosynthesis of sialyl Lewis X epitope in leukocytes and high endothelial venule cells (3). FUT8 is an α1-6 fucosyltransferase that adds a fucose to the chitobiose core of N-glycans (4). Predicted as type II transmembrane proteins and Golgi enzymes, some of the fucosyltransferases can also be found in plasma.
R&D Systems recombinant human FUTs correspond to the luminal domains. The enzymatic activity of recombinant human FUT3 was determined using a phosphatase-coupled glycosyltransferase assay (5).

References:

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5. Wu, Z.L. *et al.* (2011) Glycobiology **21**:727.

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