Recombinant Human Fucosyltransferase 7/FUT7  
Catalog Number: 6409-GT

DESCRIPTION

Source  
Chinese Hamster Ovary cell line, CHO-derived human Fucosyltransferase 7/FUT7 protein  
Ala36-Ala342 with a C-terminal 6-His tag  
Accession # Q11130

N-terminal Sequence Analysis  
Ala36

Predicted Molecular Mass  
36 kDa

SPECIFICATIONS

SDS-PAGE  
40-45 kDa, reducing conditions

Activity  
Measured by its ability to transfer fucose from GDP-fucose to fetal bovine fetuin.  
The specific activity is >175 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  
>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

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: 100 mM Tris, 10 mM MnCl₂, 5 mM CaCl₂, pH 7.5
- Recombinant Human Fucosyltransferase 7/FUT7 (rhFUT7) (Catalog # 6409-GT)
- Donor Substrate: GDP-Fucose (Sigma, Catalog # G4401), 1.6 mM stock in deionized water
- Acceptor Substrate: Fetuin, from Fetal Bovine Serum (Sigma, Catalog # F3385), 50 mg/mL 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 Coupling Phosphatase 1 to 0.1 mg/mL in Assay Buffer.
2. Prepare reaction mixture by combining 30 μL of 1.6 mM GDP-Fucose, 120 μL of 50 mg/mL Fetuin, 24 μL of 0.1 mg/mL Coupling Phosphatase 1 and 246 μL of Assay Buffer. This volume is sufficient to assay 10 wells.
3. Dilute rhFUT7 to 5 μg/mL in Assay Buffer.
4. Dilute 1 mM Phosphate Standard by adding 40 μL of the 1 mM Phosphate Standard to 360 μL of Assay Buffer for a 100 μM stock.
5. 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.
6. Load 50 μL of each dilution of the standard curve into a plate. Include a curve blank containing 50 μL of Assay Buffer.
7. Load 15 μL of the 5 μg/mL rhFUT7 into the plate. Include a Control containing 15 μL of Assay Buffer.
8. Add 35 μL of reaction mixture (step 2) to the wells, excluding the standard curve and curve blank.
9. Cover the plate with a plate sealer and incubate at 37 °C for 30 minutes.
10. Add 30 μL of the Malachite Green Reagent A to all wells. Mix briefly.
11. Add 100 μL of deionized water to all wells. Mix briefly.
12. Add 30 μL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
13. Read plate at 620 nm (absorbance) in endpoint mode.

Calculate specific activity:

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\text{Specific Activity (pmol/min/μg)} = \frac{\text{Phosphate released} (\text{nmol}) \times (1000 \text{ pmol/nmol})}{\text{Incubation time (min)} \times \text{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:

- rhFUT7: 0.075 μg
- Coupling Phosphatase 1: 0.2 μg
- Fetuin: 500 μg
- GDP-Fucose: 4000 pmol

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**

Lewis epitopes are key elements involved in the leukocyte homing and extravasation process and thus are important for lymphocyte maturation and natural defense functions. Fucose-containing glycans also play critical roles in cell signaling and development (1). More than 10 fucosyltransferases have been cloned (2). FUT1 and FUT2 are alpha 1-2 fucosyltransferases and are responsible for ABO blood-group antigen synthesis. FUT8 is an alpha 1-6 fucosyltransferase that adds a fucose to the chitobiose core of N-glycans (3). FUT3, FUT4, FUT5, FUT6, FUT7 and FUT9 are alpha 1-3 or alpha 1-4 fucosyltransferases and are responsible for Lewis antigen generation.

FUT7 plays an exclusive role for the biosynthesis of sialyl Lewis X (sLeX) epitope (NeuAc alpha 2,3Gal beta 1,4 [Fuc alpha 1,3] GlcNAc) that serves as a ligand in the E-selectin and P-selectin mediated adhesion of leukocytes to activated endothelium or platelets, and it is critical for the extravasation of immune cells (4, 5, 6). The activity of this enzyme has been measured with a phosphatase-coupled method (7).

R&D Systems Recombinant Human FUT7 has been used for the cell surface glycoengineering of several cell types. In both B cells and mesenchymal stem cells, FUT7 generated, cell surface sLeX leads to enhanced engagement with E-Selectin ligands (8, 9). In naïve regulatory T cells (Treg), engineered sLeX promotes homing to areas of inflammation in vivo (10). These studies suggest that FUT7-mediated generation of sLeX has potential to increase the efficacy of cellular-based therapeutics by enhanced targeting of cells to areas of pathology.

References: