Recombinant Human Fucosyltransferase 1/FUT1

Catalog Number: 6485-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived His26-Pro365, with an N-terminal human CD33 signal sequence and 6-His tag Accession # P19526
N-terminal Sequence Analysis	His
Predicted Molecular Mass	39 kDa

SPECIFICATIONS

SDS-PAGE	45-55 kDa, reducing conditions
Activity	Measured by its ability to transfer fucose from GDP-fucose to lactose. The specific activity is >450 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>75%, 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, NaCl and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 150 mM NaCl, 10 mM $MnCl_2$, 5 mM $CaCl_2$, pH 7.5
- Recombinant Human Fucosyltransferase 1/FUT1 (rhFUT1) (Catalog # 6485-GT)
- α-Lactose (Sigma, Catalog # L2643), 0.3 M stock in deionized water
- GDP-Fucose (Sigma, Catalog # G4401), 1.6 mM stock 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 1 mM Phosphate Standard by adding 40 µL to 360 µL of Assay Buffer for a 100 µM stock.
2. 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.
3. Dilute GDP-Fucose to 240 µM in Assay Buffer.
4. Dilute α-Lactose to 90 mM in Assay Buffer.
5. Dilute Coupling Phosphatase 1 to 6 µg/mL in Assay Buffer.
6. Prepare reaction mixture by combining equal volumes of 240 µM GDP-Fucose, 90 mM α-Lactose, and 6 µg/mL Coupling Phosphatase 1.
7. Dilute rhFUT1 to 1 µg/mL in Assay Buffer.
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 1 µg/mL rhFUT1 into the plate. Include a Control containing 25 µL of Assay Buffer.
10. Add 25 µL of reaction mixture to the wells, excluding the standard curve.
11. Cover the plate with parafilm or a plate sealer and incubate at room temperature for 30 minutes.
12. Add 30 µL of the Malachite Green Reagent A to all wells. Mix and incubate for 10 minutes at room temperature.
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:

- rhFUT1: 0.025 µg
- Coupling Phosphatase 1: 0.05 µg
- α-Lactose: 15 mM
- GDP-Fucose: 40 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 3 months from date of receipt, -70 °C as supplied. ● 1 month, -70 °C under sterile conditions after opening.

* Coomassie is a registered trademark of Imperial Chemical Industries Ltd.

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BACKGROUND

Glycans are frequently fucosylated at terminal sites. Therefore, fucose is often part of a sugar epitope with important biological function. Well known fucose-containing glycans include Lewis and ABO blood group antigens. Lewis epitopes are key elements involved in leukocyte homing and the 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, and most are Golgi-resident type II membrane proteins (2). FUT3, FUT4, FUT5, FUT6, FUT7 and FUT9 are α1-3 or α1-4 fucosyltransferases and are responsible for Lewis antigen generation (3, 4, 5). FUT8 is the only α1-6 fucosyltransferase that adds a fucose to the chitobiose core of N-glycans (6). FUT1 and FUT2 are galactoside α1-2 fucosyltransferases that generate H-antigen, the precursor for ABO blood-group antigen synthesis. While FUT2 is involved in generating soluble ABO antigen in saliva (7), FUT1 is involved in generating ABO antigen on red blood cells (8). Mutations in the FUT1 gene are the cause of the H-antigen deficient Bombay blood group (9). The activity of this enzyme has been measured with a phosphatase-coupled method (10).

References:

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