R&D SYSTEMS
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Recombinant Human Fucosyltransferase 2/FUT2

Catalog Number: 7770-GT

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived Ile35-His343, with an C-terminal 6-His tag Accession # Q10981
N-terminal Sequence Analysis	Ile35
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	40-50 kDa, reducing conditions
Activity	Measured by its ability to transfer fucose from GDP-fucose to lactose. The specific activity is >2,500 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, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 150 mM NaCl, 10 mM $MnCl_2$, 10 mM $CaCl_2$, pH 7.5
- Recombinant Human FUT2 (rhFUT2) (Catalog # 7770-GT)
- GDP-Fucose (Sigma, Catalog # G4401), 1.6 mM stock in deionized water
- α-lactose (Sigma, Catalog # L2643), 0.3 M 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 provided by the Glycosyltransferase Kit to 100 µM by adding 40 µL of standard to 360 µL of Assay Buffer. This is the first point of the standard curve.
2. Perform six additional one-half serial dilutions in Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
3. Prepare reaction mixture containg 100 mM α-lactose, 200 µM GDP-Fucose, and 4 µg/mL Coupling Phosphatase I in Assay Buffer.
4. Dilute rhFUT2 to 1.0 µg/mL in Assay Buffer.
5. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
6. Load 25 µL of the 1 µg/mL rhFUT2 into the plate. Include a Substrate Blank containing 25 µL of Assay Buffer.
7. Add 25 µL of reaction mixture (step 3) to the wells, excluding the standard curve and curve blank.
8. Cover the plate with a plate sealer and incubate at 37 °C for 20 minutes.
9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and inubate for 20 minutes at room temperature.
12. Read plate at 620 nm (absorbance) in endpoint mode.
13. 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:

- rhFUT-2: 0.025 µg
- Coupling Phosphatase I: 0.1 µg
- α-Lactose: 50 mM
- GDP-Fucose: 100 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.

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

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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. In particular, FUT2 involves in generating soluble ABO antigen in saliva; therefore it is also called secretor blood group α1-2 fucosyltransferase (7). Absence of functional FUT2 is related to Crohn's disease (8). The activity of this enzyme has been measured with a phosphatase-coupled method (9).

References:

1. Jafar-Nejad, H. *et al.* (2010) Glycobiology **20**:931.
2. Becker, D.J. *et al.* (2003) Glycobiology **13**:41R.
3. Blander, J. M. *et al.* (1999) J. Immunol. **163**:3746.
4. Natsuka, S. *et al.* (1994) J. Biol. Chem. **269**:16789.
5. Sasaki, K. *et al.* (1994) J. Biol. Chem. **269**:14730.
6. Lee, S.H. *et al.* (2006) J. Biochem. **139**:391.
7. Kelly, R.J. *et al.* (1995) J. Biol. Chem. **270**:4640.
8. McGovern, D.P. *et al.* (2010) Hum. Mol. Genet. **19**:3468.
9. Wu, Z.L. *et al.* (2011) Glycobiology **21**:727.

PRODUCT SPECIFIC NOTICES

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