Recombinant Human Fucosyltransferase 6/FUT6

Catalog Number: 8959-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Arg35-Thr359 Accession # P51993
N-terminal Sequence Analysis	Arg35
Predicted Molecular Mass	38 kDa

SPECIFICATIONS

SDS-PAGE	38-50 kDa, reducing conditions
Activity	Measured by its ability to transfer fucose from GDP-fucose to *N*-Acetyllactosamine The specific activity is >200 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	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Glycosyltransferase Activity Kit (Catalog # EA001)
- 10X Assay Buffer (supplied in kit): 250 mM Tris, 100 mM $CaCl_2$, pH 7.5
- $MnCl_2$ (supplied in kit): 100 mM
- Recombinant Human Fucosyltransferase 6/FUT6 (rhFUT6) (Catalog # 8959-GT)
- GDP-Fucose (Sigma, Catalog # G4401), 1.6 mM stock in deionized water
- Lactosamine (V-Labs, Catalog # GN204), 50 mM stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare 1X Assay Buffer containing 10 mM $MnCl_2$ by combining 10X stocks and diluting 10 fold with deionized water.
2. Dilute 1 mM Phosphate Standard provided by the Glycosyltransferase Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of 1X Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
3. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock using 1X Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
4. Prepare reaction mixture containing 0.16 mM GDP-Fucose, 0.6 mM Lactosamine, and 4 µg/mL Coupling Phosphatase 1 in 1X Assay Buffer.
5. Dilute rhFUT6 to 4 ng/µL in 1X Assay Buffer.
6. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of 1X Assay Buffer.
7. Load 25 µL of 4 ng/µL rhFUT6 into empty wells of the same plate as the curve. Include a Control containing 25 µL of 1X Assay Buffer.
8. Add 25 µL of the reaction mixture to all wells, excluding the standard curve.
9. Seal plate and incubate at 37° C for 20 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 sealed plate for 20 minutes at room temperature.
13. Read plate at 620 nm (absorbance) in endpoint mode.
14. 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:

- rhFUT6: 0.1 µg
- Coupling Phosphatase 1: 0.1 µg
- GDP-Fucose: 0.08 mM
- Lactosamine: 0.3 mM

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.

bio-techne
bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956

Recombinant Human Fucosyltransferase 6/FUT6

Catalog Number: 8959-GT

BACKGROUND

N-glycans, O-glycans and glycolipids 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 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 α1-2 fucosyltransferases and are responsible for ABO blood-group antigen synthesis. FUT8 is an α1-6 fucosyltransferase that adds a fucose to the chitobiose core of N-glycans (3). FUT3, FUT4, FUT5, FUT6, FUT7 and FUT9 are α1-3 or α1-4 fucosyltransferases and are responsible for Lewis antigen generation. Among these enzymes, FUT6 probably has the most diverse substrate specificity. It is capable of directing expression of the Lewis x (Gal β 1-->4[Fuc α1-->3]GlcNAc), sialyl Lewis x (NeuNAc α 2-->3Gal β1-->4 [Fuc α 1-->3] GlcNAc), and difucosyl sialyl Lewis x (NeuNAc α2-->3Gal β 1-->4[Fuc α 1-->3]GlcNAc β1-->3 Gal β 1-->4[Fuc α1-->3]GlcNAc) epitopes. This enzyme shares 85% amino acid sequence identity with FUT3 and 89% identity with FUT5 but differs substantially in its acceptor substrate specificity. The activity of this enzyme is measured with a phosphatase-coupled method (5).

References:

1. Jafar-Nejad, H. *et al.* (2010) Glycobiology **20**:931.
2. Becker, D.J. *et al.* (2003) Glycobiology **13**:41R.
3. Lee, S.H. *et al.* (2006) J. Biochem. **139**:391.
4. Weston, B.W. *et al.* (1992) J. Biol. Chem. **267**:24575.
5. Wu, Z.L. *et al.* (2011) Glycobilogy **21**:727.

bio-techne
bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956