Recombinant *H. pylori* Fucosyltransferase

Catalog Number: 11072-GT

DESCRIPTION

Source	*E. coli*-derived *h. pylori* Fucosyltransferase protein Met1-Tyr392, with a C-terminal 6-His tag Accession # AAD07710.1
N-terminal Sequence Analysis	Met1
Predicted Molecular Mass	46 kDa

SPECIFICATIONS

SDS-PAGE	39 kDa, under reducing conditions.
Activity	Measured by its ability to transfer fucose from GDP-fucose to *N*-Acetyllactosamine The specific activity is >1500 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Glycosyltransferase Activity Kit (Catalog # EA001)
- Assay Buffer: 25 mM Tris, 10 mM $CaCl_2$, pH 7.0
- Recombinant H. pylori Fucosyltransferase (rHp FUT) (Catalog # 11072-GT)
- Lactosamine (Dextra Laboratories, Catalog # GN204), 50 mM stock in deionized water
- GDP-Fucose (Sigma, Catalog # G4401), 1.6 mM stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute 1 mM Phosphate Standard provided by the Glycosyltransferase Activity Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
2. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
3. Prepare Reaction Mixture containing 2.4 mM Lactosamine, 0.240 mM GDP-Fucose, and 4 µg/mL Coupling Phosphatase 1 (provided in kit) in Assay Buffer.
4. Dilute rHp FUT to 1 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 1 µg/mL of rHp FUT into the plate. Include a Control containing 25 µL of Assay Buffer.
7. Start the reactions by adding 25 µL of Reaction Mixture to the wells, excluding the standard curve.
8. Incubate sealed plate 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.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate 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:

- rHp FUT: 0.025 µg
- Coupling Phosphatase 1: 0.1 µg
- Lactosamine: 1.2 mM
- GDP-Fucose: 0.12 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.

DATA

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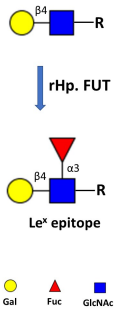
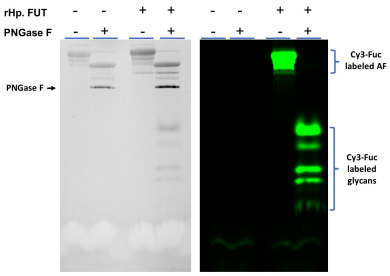
Enzyme Activity



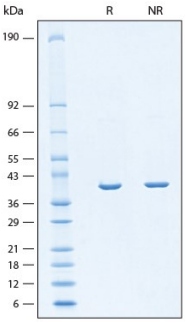
Recombinant H. pylori Fucosyltransferase Protein Enzyme Activity Diagram. Recombinant H. pylori Fucosyltransferase His-tag Protein (Catalog # 11072-GT) Enzyme Activity Diagram.

Enzyme Activity



Fluorescent glycan labeling of asialofetuin (AF) with Cy3-Fuc using rHp. FUT. Asialofetuin (4 µg) was incubated with 0.2 nmol of GDP-Cy3-Fucose (Catalog # ES401) with or without rHp. FUT (Catalog # 11072) (1.0 µg) in 25 µL of 25 mM Tris pH 7.5, 10 mM MnCl2 at 37 °C for 30 minutes. Following labeling, half of the AF was deglycosylated with PNGase F N-glycan Releasing Kit (Catalog # EA006) and resolved in 15% SDS-PAGE. Left side is the TCE image and right side is the Fluorescent image of the same gel.

SDS-PAGE



Recombinant H. pylori Fucosyltransferase Protein SDS-PAGE. 2 µg/lane of Recombinant H. pylori Fucosyltransferase Protein (Catalog # 11072-GT) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 39 kDa.

BACKGROUND

Lewis X (Le^X), a fucosylated trisaccharide glycan epitope (Galβ1,4 [Fucα1,3]GlcNAcβ) also known as CD15, and sialylated Lewis X (sLe^X) are distributed throughout eukaryotes and are determinants of many functional glycoconjugates that play central roles in numerous physiological and pathological processes (1, 2). Le^X-bearing glycans are also found in the infectious bacterium Helicobacter pylori to mask the bacterium from the host immune surveillance (3). H. pylori is the pathogen that causes peptic ulcers that can further lead to stomach cancer and gastritis. H. pylori fucosyltransferase is responsible for generating the Le^X glycans, therefore is potentially a drug target for curing peptic ulcers, gastritis and stomach cancer. In molecular terms, H. pylori fucosyltransferase is an α1,3 fucosyltransferase that shares function with human FUT4, FUT5, FUT6, and FUT9 (4). Remarkably, H. pylori fucosyltransferase can transfer IgG antibody to the glycocalyx on the surfaces of live cells when the antibody is conjugated to the enzyme's natural donor substrate GDP-Fucose (5).
The activity of this enzyme has been measured with a phosphatase coupled method (6).

References:

1. Gooi, H.C. *et al.* (1981) Nature **292**:156.
2. Phillips, M.L. *et al.* (1990) Science **250**:1130.
3. Moran, A.P. *et al.* (1996) FEMS Immunol Med Microbiol **16**:105.
4. de Vries, T. *et al.* (2001). Glycobiology **11**:119R.
5. Li, J. *et al.* (2018) ACS Cent. Sci. **4**:1633.
6. Wu, Z.L. *et al.* (2011) Glycobiology **21**:727.

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