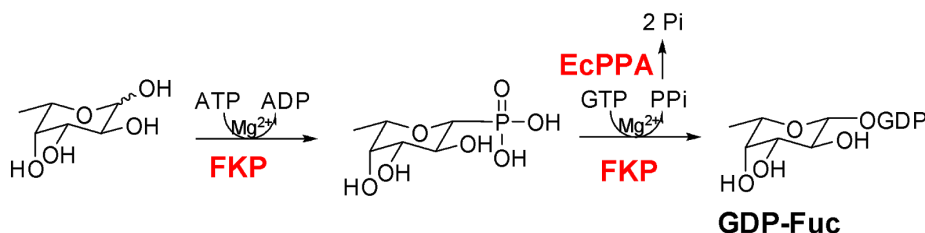




GDP-Fucose (GDP-Fuc) Kit



Notes:

- All reagents and kit components should be stored at -20 °C until use
- This kit is intended for:
- GDP-Fuc kit containing substrates (ATP, GTP, Fuc) and enzyme (FKP, PPA) is mini test kit in situ which is necessary step for large scale glycosylation.
- lower cost, continuous generation of up to 18 μ moles of nucleotide sugar *in situ* for use with sugar transferases (not included)
- conversion of sugar derivatives (not included) to the corresponding nucleotide sugar
- adding more ATP, GTP and Fuc to the reaction will continually generate large quantity of GDP-Fuc (not guaranteed) while FKP and PPA are still active

Quick start protocol

Step 1: Inspect kit contents.

- Substrate Tube A: ATP (powder ; qty 1)
- Substrate Tube B: GTP (powder ; qty 1)
- Substrate Tube C: Fucose sugar (powder ; qty 1)
- Enzyme Tube D: FKP (powder ; qty 1)
- Enzyme Tube E: EcPPA (powder ; qty 1)
- Reaction Tube F: Sterile empty tube (qty 1)
- Centrifuge all tubes briefly to pellet any material from walls of tube before opening tubes



Step 2: Assemble additional components (not included).

- Create a 20X Buffer Solution (1M Tris pH 8.0) Add 300 μ L to Reaction Tube F.
- Create a 10X Salt Solution (200mM $MgCl_2$) Add 100 μ L to Reaction Tube F.
- Obtain sterile distilled water (dH_2O) Add 100 μ L to Reaction Tube F.

Step 3: Prepare reagents.

- Add 100 μ L of dH_2O from Step 2 to Substrate Tube A. Tap gently to mix. Centrifuge briefly to pellet any insoluble material. Transfer all 100 μ L to Reaction Tube F
- Repeat with Substrate Tube B
- Repeat with Substrate Tube C. [*Note: If using a Fucose derivative instead of Fucose as the sugar substrate, skip this step. Fucose derivative not included*]
- Repeat with Enzyme Tube D [*Note: Enzymes should always be added to Reaction Tube F last*]
- Repeat with Enzyme Tube E

Step 4: Initiate nucleotide sugar reaction.

- Ensure that Reaction Tube F contain all reagents. Final reaction volume is 1000 μ L
- Incubate Reaction Tube F for 3 h in 37 °C water bath
- After 3 h, centrifuge briefly to pellet condensation and any insoluble material
- Reaction Tube F now contains GDP-Fucose for glycosylation reactions

Step 5: Glycosyltransferase reaction.

- Reaction Tube F can still actively produce GDP-Fucose *in situ*
- Add a Fucose transferase (such as $\alpha 1,3FucT$; Chemily product EN01020) and target substrate (such as $Gal\beta 4GlcNAc$) to Reaction Tube F
- Incubate Reaction Tube F in 37 °C water bath for 24 hr to initiate glycosylation of the target substrate

[*Note: Glycosylation rate may vary by transferase and target substrate*]