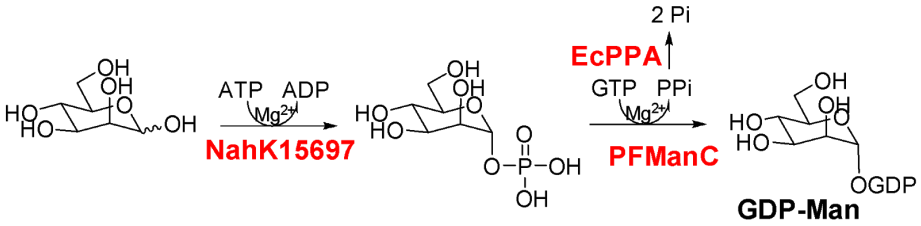
Chemily Glycoscience

NEW

GDP-Mannose (GDP-Man) Kit



Notes:

· All reagents and kit components should be stored at -20 °C until use

· This kit is intended for:

· GDP-Man kit containing substrates (GTP, Man) and enzyme (NahK15697, PFManC, PPA) is mini test kit in situ which is necessary step for large scale glycosylation.

· lower cost, continuous generation of up to 18 µmoles 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 GTP and Man to the reaction will continually generate large quantity of GDP-Man (not guaranteed) while NahK15697, PFManC and PPA are still active

Quick start protocol

Step 1: Inspect kit contents.

-Substrate Tube A: GTP (powder ; qty 1)

-Substrate Tube B: UTP (powder ; qty 1)

-Substrate Tube C: Mannose sugar (powder ; qty 1)

-Enzyme Tube D: NahK15697 (powder ; qty 1)

-Enzyme Tube E: PFManC (powder ; qty 1)

-Enzyme Tube F: EcPPA (powder ; qty 1)

-Reaction Tube G: Sterile empty tube (qty 1)

-Centrifuge all tubes briefly to pellet any material from walls of tube before opening tubes

For technical assistance: sales@chemilyus.com

Step 2: Assemble additional components (not included).

-Create a 20X Buffer Solution (1M Tris pH 8.0) Add 300µL to Reaction Tube F.

-Create a 10X Salt Solution (200mM $MgCl_2$) Add 100 µL to Reaction Tube F.

Step 3: Prepare reagents.

-Add 100 µL of dH_2O from Step 2 to Substrate Tube A. Tap gently to mix. Centrifuge briefly to pellet any insoluble material. Transfer all 100 µL to Reaction Tube G

-Repeat with Substrate Tube B

-Repeat with Substrate Tube C. *[Note: If using a Mannose derivative instead of Mannose as the sugar substrate, skip this step. Mannose derivative not included.]*

-Repeat with Enzyme Tube D *[Note: Enzymes should always be added to Reaction Tube G last]*

-Repeat with Enzyme Tube E

-Repeat with Enzyme Tube F

Step 4: Initiate nucleotide sugar reaction.

-Ensure that Reaction Tube G contain all reagents. Final reaction volume is 1000 µL

-Incubate Reaction Tube G for 3 h in 37 °C water bath

-After 3 h, centrifuge briefly to pellet condensation and any insoluble material

-Reaction Tube G now contains GDP-Mannose for glycosylation reactions

Step 5: Glycosyltransferase reaction.

-Reaction Tube G can still actively produce GDP-Mannose *in situ*

-Add a Mannose transferase and target substrate to Reaction Tube G

-Incubate Reaction Tube G 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]