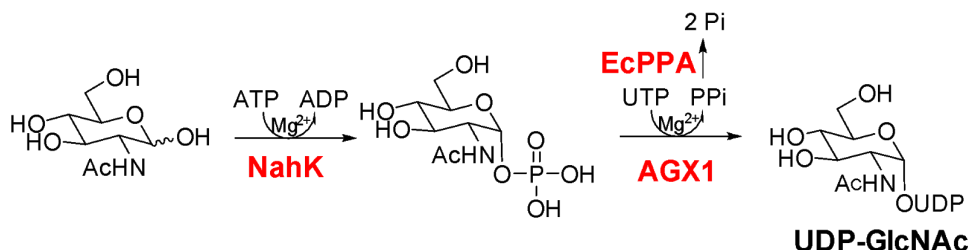




UDP-N-acetylglucosamine (UDP-GlcNAc) Kit



Notes:

- All reagents and kit components should be stored at -20 °C until use
- This kit is intended for:
- UDP-GlcNAc kit containing substrates (ATP, UTP, GlcNAc) and enzyme (NahK, AGX1, 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 ATP, UTP and GalNAc to the reaction will continually generate large quantity of UDP-GlcNAc (not guaranteed) while NahK, AGX1 and PPA are still active

Quick start protocol

Step 1: Inspect kit contents.

- Substrate Tube A: ATP (powder ; qty 1)
- Substrate Tube B: UTP (powder ; qty 1)
- Substrate Tube C: GlcNAc sugar (powder ; qty 1)
- Enzyme Tube D: NahK (powder ; qty 1)
- Enzyme Tube E: AGX1 (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.

Step 2: Assemble additional components (not included).

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

-Create a 10X Salt Solution (200mM $MgCl_2$) Add 100 μ L to Reaction Tube G.

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 G

-Repeat with Substrate Tube B

-Repeat with Substrate Tube C. *[Note: If using a GlcNAc derivative instead of GlcNAc as the sugar substrate, skip this step. GalNAc 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 μ L

-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 UDP-GlcNAc for glycosylation reactions

Step 5: Glycosyltransferase Reaction.

-Reaction Tube G can still actively produce UDP-GlcNAc

-Add a GlcNAc transferase (such as LgtA ; Chemily product EN01030) and target substrate (such as lactose) to Reaction Tube G

-Incubate Reaction Tube G in 37 °C water bath for 24 hr to initiate glycosylation of the target substrate



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

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