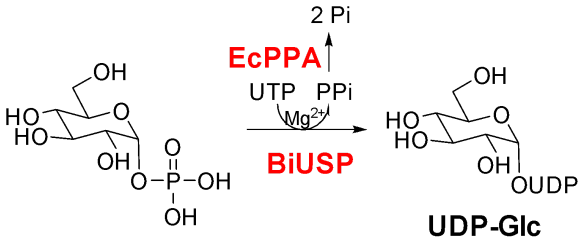
Chemily Glycoscience

NEW

UDP-Glucose (UDP-Glc) Kit



Notes:

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

· This kit is intended for:

· UDP-Glc kit containing substrates (UTP, Glc-1-P) and enzyme (BiUSP, 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 UTP and Glc-1-P to the reaction will continually generate large quantity of UDP-Glc (not guaranteed) while BiUSP and PPA are still active

Quick start protocol

Step 1: Inspect kit contents.

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

-Substrate Tube B: Glc-1-P (powder ; qty 1)

-Substrate Tube C: BiUSP (powder ; qty 1)

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

-Enzyme Tube E: 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).

For technical assistance: sales@chemilyus.com

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

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 E

-Repeat with Substrate Tube B *[Note: If using a Glc-1-P derivative instead of Glc-1-P as the sugar substrate, skip this step. Glc-1-P derivative not included.]*

-Repeat with Substrate Tube C *[Note: Enzymes should always be added to Reaction Tube E last]*

-Repeat with Enzyme Tube D

Step 4: Initiate nucleotide sugar reaction.

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

-Incubate Reaction Tube E for 6 h in 37 °C water bath

-After 6 h, centrifuge briefly to pellet condensation and any insoluble material

-Reaction Tube E now contains UDP-Glc for glycosylation reactions

Step 5: Glycosyltransferase Reaction.

-Reaction Tube E can still actively produce UDP-Glc

-Add a Glc transferase and target substrate to Reaction Tube E

-Incubate Reaction Tube E 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]