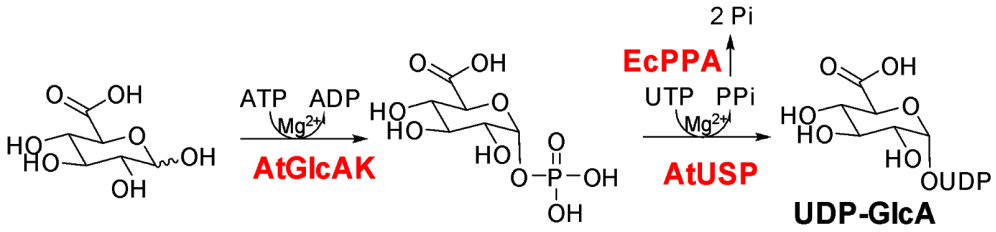
Chemily Glycoscience

NEW

UDP-glucuronic acid (UDP-GlcA) Kit



Notes:

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

· This kit is intended for:

· UDP-GlcA kit containing substrates (ATP, UTP, GlcA) and enzyme (AtGlcAK, AtUSP, 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 GlcA to the reaction will continually generate large quantity of UDP-GlcA (not guaranteed) while AtGlcAK, AtUSP 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: GlcA sugar (powder ; qty 1)

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

-Enzyme Tube E: AtUSP (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 300 µL to Reaction Tube G.

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

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 GlcA derivative instead of GlcA 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 µL

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

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

-Reaction Tube G now contains UDP-GlcA for glycosylation reactions

Step 5: Glycosyltransferase Reaction.

-Reaction Tube G can still actively produce UDP-GlcA

-Add a GlcA transferase (such as PmHAS ; Chemily product EN01025) and target substrate (such as GlcNAc) 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]

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