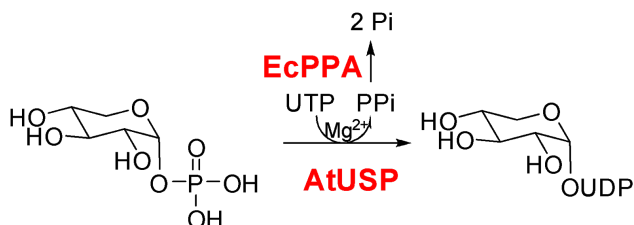




## UDP-Xylose (UDP-Xyl) Kit



### Notes:

- All reagents and kit components should be stored at -20 °C until use
- This kit is intended for:
- UDP-Xyl kit containing substrates (UTP, Xyl-1-P) and enzyme (AtUSP, PPA) is mini test kit *in situ* which is necessary step for large scale glycosylation.
- lower cost, continuous generation of up to 18  $\mu$ 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 UTP and Xyl-1-P to the reaction will continually generate large quantity of UDP-Xyl (not guaranteed) while AtUSP and PPA are still active

## Quick start protocol

### Step 1: Inspect kit contents.

- Substrate Tube A: UTP (powder ; qty 1)
- Substrate Tube B: Xyl-1-P (powder ; qty 1)
- Substrate Tube C: AtUSP (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.



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### **Step 2: Assemble additional components (not included).**

- Create a 20X Buffer Solution (1M Tris pH 8.0)      Add 300  $\mu\text{L}$  to Reaction Tube E.
- Create a 10X Salt Solution (200mM  $\text{MgCl}_2$ )      Add 100  $\mu\text{L}$  to Reaction Tube E.
- Obtain sterile distilled water ( $\text{dH}_2\text{O}$ )      Add 200  $\mu\text{L}$  to Reaction Tube E.

### **Step 3: Prepare reagents.**

- Add 100  $\mu\text{L}$  of  $\text{dH}_2\text{O}$  from Step 2 to Substrate Tube A. Tap gently to mix. Centrifuge briefly to pellet any insoluble material. Transfer all 100  $\mu\text{L}$  to Reaction Tube E
- Repeat with Substrate Tube B . *[Note: If using a Xyl-1-P derivative instead of Xyl-1-P as the sugar substrate, skip this step. Xyl-1-P derivative not included.]*
- Repeat with Enzyme 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  $\mu\text{L}$
- Incubate Reaction Tube E for 12 h in 37  $^\circ\text{C}$  water bath
- After 12 h, centrifuge briefly to pellet condensation and any insoluble material
- Reaction Tube E now contains UDP-Xyl for glycosylation reactions

### **Step 5: Glycosyltransferase Reaction.**

- Reaction Tube E can still actively produce UDP-Xyl
- Add a Xyl transferase and target substrate to Reaction Tube E
- Incubate Reaction Tube E in 37  $^\circ\text{C}$  water bath for 24 hr to initiate glycosylation of the target substrate

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