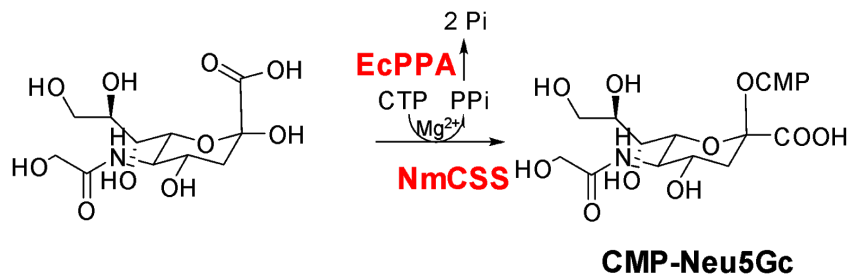




CMP-N-glycolylneuraminic acid (CMP-Neu5Gc) Kit



Notes:

- All reagents and kit components should be stored at -20 °C until use
- This kit is intended for:
- CMP-Neu5Gc kit containing substrates (Neu5Gc, CTP) and enzyme (NmCSS, PPA) is mini test kit *in situ* which is necessary step for large scale glycosylation.
- lower cost, continuous generation of up to 18 μ 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 Neu5Gc and CTP to the reaction will continually generate large quantity of CMP-Neu5Gc (not guaranteed) while NmCSS and PPA are still active

Quick start protocol

Step 1: Inspect kit contents.

- Substrate Tube A: CTP (powder ; qty 1)
- Substrate Tube B: Neu5Gc sugar (powder ; qty 1)
- Enzyme Tube C: NmCSS (powder ; qty 1)
- Enzyme Tube D: EcPPA (powder ; qty 1)
- Reaction 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 μ L to Reaction Tube.
- Create a 10X Salt Solution (200mM $MgCl_2$) Add 100 μ L to Reaction Tube.
- Obtain sterile distilled water (dH_2O) Add 200 μ L to Reaction Tube.

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 E
- Repeat with Substrate Tube B. [*Note: If using a Neu5Gc derivative instead of Neu5Gc as the sugar substrate, skip this step. Neu5Gc derivative not included*]
- Repeat with Enzyme Tube C [*Note: Enzymes should always be added to Reaction Tube last*]
- Repeat with Enzyme Tube D

Step 4: Initiate nucleotide sugar reaction.

- Ensure that Reaction Tube contain all reagents. Final reaction volume is 1000 μ L
- Incubate Reaction Tube F for 2 h in 37 $^{\circ}$ C water bath
- After 2 h, centrifuge briefly to pellet condensation and any insoluble material
- Reaction Tube now contains CMP-Neu5Gc for glycosylation reactions

Step 5: Glycosyltransferase reaction.

- Reaction Tube can still actively produce CMP-Neu5Gc *in situ*
- Add a Neu5Gc transferase (such as PmST1 ; Chemily product EN01002) and target substrate (such as lactose) to Reaction Tube
- Incubate Reaction Tube in 37 $^{\circ}$ C water bath for 4 hr to initiate glycosylation of the target substrate

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