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CMP-N-Acetylneuraminic Acid Synthetase

recombinant from E.coli

CMP-N-acetylneuraminic acid synthetase (EC 2.7.7.43) catalyzes the formation of CMP-N-acetylneuraminic acid (CMP-NANA) and inorganic pyrophosphate from CTP and N-acetylneuraminic acid. It is useful for production of CMP-NANA for use as a donor molecule in sialic acid transferase reactions and in coupled transferase reactions.

Product Code: GE 11

Specifications

Activity: 2.5 U/mg 9 U/mL

Storage: Store at 4°C.

Formulation: Ammonium sulfate precipitate in 1 mM sodium phosphate pH 7.5. Resuspend precipitated enzyme before sampling.

Stability: Stable at least 12 months when stored properly. Several days exposure to ambient temperatures will not reduce activity.

Product Description

Molecular weight: 46,000 Daltons

Specificity: CMP-N-acetylneuraminic acid synthetase will activate N-glycolylneuraminic acid as well as many other derivatives of N-acetylneuraminic acid (see ref 2).

Purity: CMP-N-acetylneuraminic acid synthetase is tested for contaminating protease as follows: 10 µg of denatured BSA is incubated for 24 hours at 37°C with 2 mL of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

The production strain of E. coli has been extensively tested and does not produce any detectable glycosidases.

Reagents

Reaction buffer is 1M Tris Base pH 9.5

Activity definition: One unit CMP-N-acetylneuraminic acid synthetase is defined as the amount that will produce one µmole of CMP-NANA per minute at 37°C and pH 9.5 from CTP and N-acetylneuraminic acid both at 8 mM.

Suggestions for use:

20 uMole production of CMP-NANA

Mix in a 1.5 mL centrifuge tube:

200 uL 100 mM CTP in 20 mM Tris-HCl pH 7.5

6.2 mg N-acetylneuraminic acid (20 uMoles)

150 uL 1 M Tris Base

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50 uL 1 M $MgCl_2$

20 uL 1 N NaOH

Add 20 uL CMP-N-acetylneuraminic acid synthetase suspension

Incubate at 37°C

After 10 minutes, a white precipitate will form.

Add 20 uL of 1 N NaOH. Spin down the precipitate.

Incubate an additional 10 min

Another precipitate will form .

Add an additional 20 uL of 1 N NaOH. Spin down the precipitate. Continue to incubate.

One more precipitate will form Add 20 uL of 1 N NaOH. Spin down the precipitate

If no more precipitate forms upon futher incubation, the reaction is complete.

Spin once more and aliquot 100 ul(5 nMoles) of solution into 4 1.5 mL centrifuge tubes.

Add 0.9 mL ethanol and cool in freezer. A white precipitate will form.

Centrifuge the precipitate. Carefully remove supernatant.

Dry the CMP-NANA in a speedvac.

Store at 20°C

Note: Addition of NaOH is necessary to keep the pH between 8.5-9.5. Check with pH paper. The formation of pyrophosphoric acid rapidly lowers the pH.

References:

1. Vann, W.F., Silver, R.P., Abeijon, C., Chang, K., Aaronson, W., Sutton,A., Finn, C.W., Lindner, W. and Kotsatos, M. (1987) Purification, properties and genetic location of *Escherichia coli cytidine* 5'-monophosphate N-acetylneuraminic acid synthetase *J. Biol. Chem*. *262*, 17556-17562.

2. Shames, S.I., Simon, E. S., Christopher, C.W., Schmid, W, Whitesides, G.G. and Yang, L. (1991) CMP-N-acetylneuraminic acid synthetase of *Escherichia coli*: high level expression, purification and use in the enzymatic synthesis of CMP-N-acetylneuraminic acid and CMP-neuraminic acid derivatives. *Glycobiology* **1**, 187-191

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