

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.

Specifications

> 2 U/mg > 9 U/mL

Formulation

1 mM sodium phosphate pH 7.5, sterile filtered

Stability

Stable at least 12 months when stored at 4°C. Several days exposure to ambient temperature will not reduce activity.

Product Description

Molecular Weight

46,000 Daltons

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 µL 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.

Specificity

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

Reagents

- 5X Reaction buffer is 200 mM Tris-HCl pH 9.5
200 mM MgCl₂

Activity Definition:

One unit CMP-N-acetylneuraminic acid synthetase is defined as the amount that will produce one micro-mole 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

Large scale production of CMP-NANA.

From ref 2.

2.2 g CTP (3.1 mM)

1 g N-acetylneuraminic acid (3.2 mM)

8 U CMP-N-acetylneuraminic acid synthetase in 50 mL of 50 mM Tris HCl pH 8.5, 50 mM MgCl₂

Incubate for 3.5 hr at 22°C with stirring and maintain the pH at 8.5 with addition of 1 N NaOH.

Filter out any precipitate. To the supernatant, add 9 volumes ethanol and collect the precipitate by centrifugation at 10,000 x g for 10 minutes. Dry under vacuum. Yield ~ 90% at 95% purity.

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.

Order Information

Catalog No.	Product Description	Package Size	Temperature
100380-1	CMP-N-acetylneuraminic Acid Synthetase <i>recombinant from E.coli</i>	60 µL	4°C

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