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N-Acetylneuraminic Acid Aldolase

N-Acetylneuraminic Acid Aldolase (N-Acetylneuraminate pyruvate lyase, EC 4.1.3.3) is found in several bacterial strains which use the reverse reaction to degrade N-acetylneuraminic acid (sialic acid). N-acetylneuraminic Acid Aldolase catalyzes the reversible reaction:

N-acetylneuraminic acid \rightleftharpoons N-acetylmannosamine + Pyruvic acid

The forward reaction is particularly useful for the determination of N-acetylneuraminic acid concentrations by quantitatively converting it to N-acetylmannosamine and pyruvate. Since N-acetylneuraminic acid is both negatively charged and not a reducing sugar, its direct analysis is more difficult than conventional sugars. N-acetylmannosamine, however, can be assayed as a conventional reducing sugar by various techniques such as fluorescent dye or radioactive labeling.

Alternatively, the pyruvic acid generated in the reaction can be assayed using enzymes such as Lactic Dehydrogenase, coupled to NADH oxidation, to reduce pyruvate. NADH oxidation can be spectrophotometrically quantitated. Another method uses pyruvate oxidase to generate hydrogen peroxide which is measured colorimetrically.

N-acetylneuraminic Acid Aldolase is produced from a *Escherichia coli* strain K1 clone.

Product Code: GE 10

Specifications

Activity: ~15 U/mg, ~100 U/mL

Storage: Store at 4°C.

Formulation: The enzyme is provided as a sterile solution in 20 mM Tris-HCl pH 7.5.

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

Product Description

Molecular weight: 32,000 Daltons

Purity: Each lot of N-acetylneuraminic Acid Aldolase is tested for contaminating NADH Oxidase by incubating the enzyme for 24 hours at 37°C with the appropriate substrate; the detection limit of this assay is 5 µU/mL (IUB). A passing lot will have no detectable activity.

For the protease assay, 10 µg of denatured BSA is incubated for 24 hr with 2 µl of enzyme. Analysis of the BSA band after SDS-PAGE should show no evidence of degradation.

Specificity: Cleaves N-acetylneuraminic acid and many analogs (i.e. N-glycolylneuraminic acid).

Assay

One unit of N-acetylneuraminic Acid Aldolase will release one umole of pyruvate from N-acetylneuraminic acid in one minute at 37°C, pH 7.5 at a substrate concentration of 20 mM. Pyruvate production is monitored by the

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oxidation of NADH in the presence of Lactic Dehydrogenase.

References

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- Lilley, G.G., M. von Itzstein and N. Ivancic. High-Level Production and Purification of Escherichia coli N-Acetylneuraminic Acid Aldolase (EC 4.1.3.3) **Protein Expression and Purification** **3**:434-440 (1992).
- Ohta, Y., K. Watanabe and A. Kimura. Complete Sequence of E. coli N-acetylneuraminate lyase. **Nucleic Acids Res.** **13**:8843-8852(1985).

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This product is intended for in vitro research only.

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