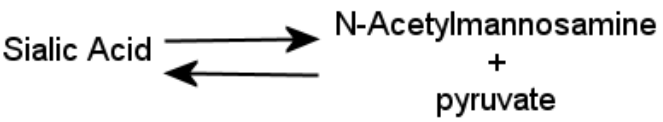

Sialic Acid Aldolase

Source
produced from a *Escherichia coli* strain K1 clone.

Catalog Number
E-ALD01

Contents
1 vial:
Sialic Acid Aldolase- 60 µL (6 U)
in 20 mM tris-HCl, pH 7.5

Specific Activity 15 U/mg
Activity 100 U/ml

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

Specificity
Sialic Acid Aldolase (N-Acetylneuraminate pyruvate lyase, EC 4.1.3.3) catalyzes the reversible reaction of sialic acid (N-acetylneuraminic acid) to N-acetylmannosamine and pyruvic acid.

Molecular weight ~32,000 daltons

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

Storage
Store enzyme at 4°C. Do not freeze.

Applications
The enzyme is found in several bacterial strains which use the reverse reaction to degrade N-acetylneuraminic acid (sialic acid).The forward reaction is particularly useful for the determination of sialic acid concentrations by quantitatively converting it to N-acetylmannosamine and pyruvate.

Since sialic acid is both negatively charged and a non-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 flourescent 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.

In addition to free neuraminic acid, N-acetylneuraminic Acid Aldolase can be used to determine the total amount of neuraminic acid in:
Glycoproteins
Cell surfaces
Polysialic acids
Capsular Polysaccharides

by first digesting the whole cells, glycoprotein or polysaccharide with QA-Bio Sialidase Au (E-S001), and then determining total N-acetylneuraminic acid

E-ALD01 Sialic Acid Aldolase

Specifications - Protocol

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Activity
One unit of N-acetylneuraminic Acid Aldolase will release one µmole 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 oxidation of NADH in the presence of Lactic Dehydrogenase.

Purity
Each lot of N-acetylneuraminic Acid Aldolase is tested for contaminating NADH Oxidase by incubating the enzyme for 24 hours at 37C 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 hours with 2 µl of enzyme. Analysis of the BSA band after SDS-PAGE should show no evidence of degradation.

Directions for use
Assay Setup
First, obtain an approximation of the amount of sialic acid (NANA) in the sample to be quantitated (from the literature or gel analysis after neuraminidase treatment). Otherwise run a series of dilutions to determine the quantity of sample to be assayed. In order to meet the volumetric requirements of the assay, diluted samples may be concentrated

Assay of Free Sialic Acid Overview
NANA is converted to pyruvic acid by N-acetylneuramanic acid Aldolase, and then subsequently treated with Lactic Dehydrogenase to form lactic acid with the oxidation of β–NADH to β-NAD (monitored spectrophotometrically).

Additonal Reagents
NANA Sample - The sample may be in solution or a lyophilizate that when added to the Reaction Buffer should contain between 1 and 200 nmoles of NANA.

25 mM Tris Reaction Buffer

Lactic Dehydrogenase

β-NADH Solution - Just prior to use, add 256 µl Tris Reaction Buffer to one of the vials of β-NADH (supplied with the kit) to make a 0.01 M solution (A340 = 62.2). Store at 40C in the dark for not more than 1 month. Discard if A340 drops 20%, or solution turns yellow.

Procedure
1. Add sample to Tris Reaction Buffer so that the final volume is 980 µl and equilibrate to 37°C.
2. Add 1 µl Aldolase. Incubate in a 37°C water bath for a minimum of 10 minutes.
3. Pipette entire contents into cuvette and blank the spectrophotometer. Add 20 µl b-NADH Solution and mix by inverting several times.
4. Incubate in a 37C water bath for a minimum of 10 minutes.
5. Read and record the $A_{initial}$. The A340 should read ~1.25.
6. Incubate in a 37EC water bath for a minimum of 10 minutes.
7. Read and record the A_{final}. Calculate the nmoles of NANA:

nmoles NANA= $((A_{initial}-A_{final})x1000)/6.22$

References
Kolisis, F.N. An immobilized bienzyme system for assay of sialic acid. Biotechnol Appl Biochem 8:).

Simpson, H., G. D. Ghusney, M. A. Crook and J. C. Pickup. Serum sialic acid enzymatic assay based on microtitre plates: application for measuring capillary serum sialic acid concentrations. Br J Biomed Sci 50:164-167 (1993).

Sugahara K, K. Sugimoto, O. Nomura and T. Usui. Enzymatic assay of serum sialic acid. Clin Chim Acta 108:).

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).

Warranties and liabilities
QA-Bio warrants that the above product conforms to the specifications described herein. Should the product fail for reasons other than through misuse QA-Bio will, at its option, replace free of charge or refund the purchase price. This warranty is exclusive and QA-Bio makes no other warrants, expressed or implied, including any implied conditions or warranties of merchantability or fitness for any particular purpose.

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