Enzymatic Assay of N-ACETYLNEURAMINIC ACID ALDOLASE (EC 4.1.3.3)

PRINCIPLE:

NANA $\xrightarrow{\text{NANA-Aldolase}}$ N-Acetyl-D-Mannosamine + Pyruvate

Pyruvate + ß-NADH $\xrightarrow{\text{L-Lactic Dehydrogenase}}$ L-Lactate + ß-NAD

Abbreviations used:
NANA = N-Acetylneuraminic Acid
NANA-Aldolase = N-Acetylneuraminic Acid Aldolase
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 37°C, pH = 7.2, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 20 mM Potassium Phosphate Buffer, pH 7.2 at 37°C
(Prepare 200 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379. Adjust to pH 7.2 at 37°C with 1 M KOH.)

B. 65 mM N-Acetylneuraminic Acid Solution (NANA)
(Prepare 2 ml in Reagent A using N-Acetylneuraminic Acid, Prod. No. A-2751.)

C. 1.28 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Dissolve the contents of one 5 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt,
Stock No. 340-105, in the appropriate volume of Reagent A. **PREPARE FRESH.**)

D. L-Lactic Dehydrogenase Solution (LDH)
(Immediately before use, prepare a solution containing 500 units/ml in cold Reagent A using L-Lactic Dehydrogenase, Prod. No. L-2500.)

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REAGENTS: (continued)

E. N-Acetylneuraminic Acid Aldolase Enzyme Solution (Immediately before use, prepare a solution containing 0.3 - 0.4 unit/ml of N-Acetylneuraminic Acid Aldolase in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent A (Buffer)	2.50	2.55
Reagent B (NANA)	0.20	0.20
Reagent C (ß-NADH)	0.30	0.30
Reagent D (LDH)	0.02	0.02

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Enzyme Solution)	0.05	-------

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes. Obtain the $r\ A_{340nm}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{r\ A_{340nm}/\text{min Test} - r\ A_{340nm}/\text{min Blank}}{(6.22)\ (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm RM = Reaction Mix

UNIT DEFINITION:

One unit will release 1.0 µmole of pyruvate from NANA per minute at 37°C at pH 7.2.

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FINAL ASSAY CONCENTRATION:

In a 3.07 ml reaction mix, the final concentrations are 20 mM potassium phosphate, 4.2 mM N-acetylneuraminic acid, 0.12 mM ß-NADH, 10 units L-lactic dehydrogenase and 0.015 - 0.02 unit N-acetylneuraminic acid aldolase.

REFERENCE:

Comb, D.G. and Roseman, S. (1960) *Journal of Biological Chemistry* **235**, 2529.

NOTES:

1. Unit Definition for Lactic Dehydrogenase: One unit will reduce 1.0 µmole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.

2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.