

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

### PRINCIPLE:

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

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

Abbreviations used:

NANA = N-Acetylneuraminic Acid

NANA-Aldolase = N-Acetylneuraminic Acid Aldolase

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

**CONDITIONS:** T = 37°C, pH = 7.7,  $A_{340\text{nm}}$ , Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

### REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 7.7 at 37°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379. Adjust to pH 7.7 at 37°C with 1 M KOH.)
- B. 20 mM N-Acetylneuraminic Acid Solution (NANA)  
(Prepare 25 ml in Reagent A using N-Acetylneuraminic Acid, Prod. No. A-2751.)
- C. 14 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form Solution ( $\beta$ -NADH)  
(Dissolve the contents of one 10 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Stock No. 340-110, in the appropriate volume of deionized water.  
**PREPARE FRESH.**)

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**REAGENTS:** (continued)

- D. L-Lactic Dehydrogenase Solution (LDH)  
(Immediately before use, prepare a solution containing 2750 units/ml in cold Reagent A using L-Lactic Dehydrogenase, Prod. No. L-2500)
  
- E. N-Acetylneuraminic Acid Aldolase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.2 - 0.4 unit/ml of N-Acetylneuraminic Acid Aldolase in cold Reagent A.)

**PROCEDURE:**

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

		<u>Test</u>	<u>Blank</u>
Reagent B (NANA)	2.80	2.80	
Reagent C ( $\beta$ -NADH)		0.10	0.10
Reagent D (LDH)		0.01	0.01

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

Reagent A (Buffer)	-----		0.10
Reagent E (Enzyme Solution)		0.10	-----

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

**CALCULATIONS:**

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

6.22 = Millimolar extinction coefficient of  $\beta$ -NADH at 340 nm RM = Reaction Mix

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### UNIT DEFINITION:

One unit will release 1.0  $\mu$ mole of pyruvate from NANA per minute at 37°C at pH 7.7.

### FINAL ASSAY CONCENTRATION:

In a 3.01 ml reaction mix, the final concentrations are 97 mM potassium phosphate, 19 mM N-acetylneuraminic acid, 0.47 mM  $\beta$ -NADH, 27.5 units lactic dehydrogenase and 0.02 - 0.04 unit N-acetylneuraminic acid aldolase.

### REFERENCE:

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

### NOTES:

1. Unit Definition for L-Lactic Dehydrogenase: One unit will reduce 1.0  $\mu$ mole 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.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**