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: $T = 37^\circ C$, pH = 7.7, $A_{340nm}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 7.7 at $37^\circ C$
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379. Adjust to pH 7.7 at $37^\circ C$ 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (NANA)</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent C (β-NADH)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (LDH)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

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:

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

6.22 = Millimolar extinction coefficient of β-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:


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.