SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of L-LACTIC DEHYDROGENASE¹ (EC 1.1.1.27)

PRINCIPLE:

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

Abbreviations used:

\[\beta\text{-NADH} = \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form}\]
\[\beta\text{-NAD} = \beta\text{-Nicotinamide Adenine Dinucleotide, Oxidized Form}\]

CONDITIONS: \(T = 37^\circ\text{C}, \text{pH} = 7.5, A_{340\text{nm}}, \text{Light path} = 1\ \text{cm}\)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Sodium Phosphate Buffer, pH 7.5 at 37°C
   (Prepare 200 ml in deionized water using Sodium Phosphate, Anhydrous, Monobasic, Sigma Prod. No. S-0751. Adjust to pH 7.5 at 37°C with 1 M NaOH.)

B. 0.13 mM \(\beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (}\beta\text{-NADH})\)
   (Dissolve the contents of a 1 mg vial of \(\beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-101, in the appropriate volume of cold Reagent A or prepare 10 ml in cold Reagent A using \(\beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. PREPARE FRESH.}\))

C. 34 mM Sodium Pyruvate Solution (Pyruvate)
   (Prepare 1.0 ml in cold Reagent A using Pyruvic Acid, Sodium Salt, Sigma Prod. No. P-2256.)

D. 1.0% (w/v) Bovine Serum Albumin Solution (BSA)
   (Prepare 50 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503. PREPARE FRESH)
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REAGENTS: (continued)

E. L-Lactic Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 0.75 unit/ml of L-Lactic Dehydrogenase in cold Reagent D.)

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 (β-NADH)</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent C (Pyruvate)</td>
<td>0.10</td>
<td>0.10</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 D (BSA)</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 $\Delta A_{340\text{nm}}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{\left(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank}\right)(3)(\text{df})}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay

$\text{df}$ = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADH at 340 nm

0.1 = Volume (in milliliters) of enzyme used

Units/mg solid = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}

Units/mg protein = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}
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UNIT DEFINITION:

One unit will reduce 1.0 µmole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 100 mM sodium phosphate, 0.12 mM β-nicotinamide adenine dinucleotide, 1.1 mM pyruvate, 0.03% (w/v) bovine serum albumin, and 0.025 - 0.075 unit L-lactic dehydrogenase.

REFERENCES:


NOTES:

1. This assay procedure is to be used to assay L-Lactic Dehydrogenase, Sigma Prod. Nos.: L-0133, L-0377, L-1006, L-2625, L-7525, L-2889, L-7755, L-9126, L-9382, and L-9889.
2. This assay is based on the cited reference.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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