SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of ALCOHOL DEHYDROGENASE, NADP⁺ DEPENDENT (EC 1.1.1.2)

PRINCIPLE:

2-Propanol + β-NADP Alcohol Dehydrogenase → Acetone + β-NADPH

Abbreviations used:

β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 40°C, pH = 7.8, A₃₄₀nm, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 7.8 at 40°C
   (Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253.
   Adjust to pH 7.8 at 40°C with 1 M NaOH.)

B. 15 mM β-Nicotinamide Adenine Dinucleotide Phosphate Solution (β-NADP)
   (Prepare 2.5 ml in Reagent A using β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505 or dissolve the contents of one 10 mg vial of β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-310, in an appropriate volume of Reagent A. PREPARE FRESH.)

C. 1.5 M 2-Propanol Solution (2-Prop)
   (Prepare 10 ml in Reagent A using Isopropanol, Anhydrous, Sigma Stock No. 405-7.)

D. Alcohol Dehydrogenase, NADP⁺ Dependent Enzyme Solution
   (Immediately before use, prepare a solution containing 0.10 - 0.40 unit/ml of Alcohol Dehydrogenase, NADP⁺ Dependent in cold Reagent A.)
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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 A (Buffer)</td>
<td>2.40</td>
<td>2.60</td>
</tr>
<tr>
<td>Reagent B (β-NADP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (2-Prop)</td>
<td>0.30</td>
<td>0.30</td>
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</tbody>
</table>

Mix by inversion and equilibrate to 40°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution) 0.20

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

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/min \text{ Test} - \Delta A_{340nm}/min \text{ Blank})(3)(df)}{(6.22)(0.2)}
\]

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm
0.2 = Volume (in milliliter) of enzyme used

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

\[
\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}
\]

UNIT DEFINITION:

One unit will oxidize 1.0 μmole of 2-propanol to acetone per minute at pH 7.8 at 40°C in the presence of β-NADP⁺.
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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 100 mM Tris, 0.5 mM β-nicotinamide adenine dinucleotide phosphate, 150 mM 2-propanol and 0.02 - 0.08 unit alcohol dehydrogenase, β-NADP⁺ dependent.

REFERENCES:


NOTES:

1. This assay is based on the cited references.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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