SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of SACCHAROPINE DEHYDROGENASE, NAD⁺,
Lysine Forming
(EC 1.5.1.7)

PRINCIPLE:

L-Lysine + α-Ketoglutaric + β-NADH Saccharopine Dehydrogenase > Saccharopine + β-NAD

Abbreviations used:
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS:  T = 25°C, pH = 6.8, A₃₄₀nm, Light path = 1 cm

METHOD: Continuous Spectrophotometric Method

REAGENTS:

A. 100 mM Potassium Phosphate Buffer with
1 mM Ethylenediaminetetraacetic Acid (EDTA), pH 6.8 at
25°C
(Prepare 100 ml in deionized water using Potassium
Phosphate, Monobasic, Prod. No. P-5379 and
Ethylenediaminetetraacetic Acid, Tetrasodium Hydrate,
Hydrate, Sigma Stock No. ED495. Adjust to pH 6.8 at 25°C
with 1 M NaOH.)

B. 0.23 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form
(β-NADH)
(Prepare 50 ml in Reagent A using β-Nicotinamide Adenine
Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod.
No. N-8129.)

C. 79.8 mM α-Ketoglutarate Solution
(Prepare 1.0 ml in Reagent A using α-Ketoglutaric Acid,

D. 300 mM L-Lysine Solution (L-Lysine)
(Prepare 10 ml in Reagent A using L-Lysine
Monohydrochloride, Prod. No. L-5626.)

E. Saccharopine Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing
0.1 - 0.5 units/ml of Saccharopine Dehydrogenase 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 B (β-NADH)</td>
<td>2.75</td>
<td>2.75</td>
</tr>
<tr>
<td>Reagent C (α-Ketoglutarate)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (L-Lysine)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340 nm}$ 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 E (Enzyme Solution)</td>
<td>0.10</td>
<td>-----</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>-----</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

CALCULATIONS:

$$\text{Unit/mg enzyme} = \frac{(\Delta A_{340 nm}/\text{min Test} - \Delta A_{340 nm} \text{ Blank})(3.05)(df)}{(6.22)(0.1)}$$

3.05 = Volume (in milliliters) of assay
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}}

UNIT DEFINITION:

One unit will catalyze the conversion of 1.0 μmole of L-lysine and α-ketoglutaric acid to saccharopine per minute at pH 6.8 at 25°C.
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FINAL ASSAY CONCENTRATION:

In a 3.05 ml reaction mix, the final concentrations are 100 mM potassium phosphate, 1 mM ethylenediaminetetraacetic acid, 0.21 mM β-icotinamide adenine dinucleotide, reduced form, 2.6 mM α-ketoglutaric acid, 9.8 mM L-lysine, and 0.01 - 0.05 units saccharopine dehydrogenase.

REFERENCES:


NOTES:

1. Precipitate and refrigerate vials containing 14.7 mg of α-ketoglutaric acid. Dissolve each vial with 1.0 ml of Reagent A. PREPARE FRESH, STORE ON ICE.)

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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