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

PRINCIPLE:

L-Lysine + a-Ketoglutaric + ß-NADH $\xrightarrow{\text{Saccharopine Dehydrogenase}}$ Saccharopine + ß-NAD

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

CONDITIONS: T = 25°C, pH = 6.8, A_{340nm}, 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. ED4S. Adjust to pH 6.8 at 25°C with 1 M NaOH.)

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

C. 79.8 mM a-Ketoglutarate Solution
(Prepare 1.0 ml in Reagent A using a-Ketoglutaric Acid, Monopotassium Salt, Prod. No. K-2000. **PREPARE FRESH.**)[1]

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:

	Test	Blank
Reagent B (ß-NADH)	2.75	2.75
Reagent C (a-Ketoglutarate)	0.10	0.10
Reagent D ($_L$-Lysine)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

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

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

CALCULATIONS:

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

3.05 = Volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.1 = Volume (in milliliters) 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 catalyze the conversion of 1.0 µmole of

L-lysine and a-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 ß-icotinamide adenine dinucleotide, reduced form, 2.6 mM a-ketoglutaric acid, 9.8 mM L-lysine, and 0.01 - 0.05 units saccharopine dehydrogenase.

REFERENCES:

(1978) Ogawa and Fujioka, *J. Biol. Chem.* **253**, 3666.

NOTES:

1. Preweigh and refrigerate vials containing 14.7 mg of a-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.