

**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, 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 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, 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 (β-NADH)	2.75	2.75
Reagent C (α-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 r A_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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

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

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

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 β -nicotinamide adenine dinucleotide, reduced form, 2.6 mM α -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 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.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.