Enzymatic Assay of SUCCINIC SEMIALDEHYDE DEHYDROGENASE (EC 1.2.1.16)

PRINCIPLE:

Succinic Semialdehyde + NADP + H_2O $\xrightarrow{SSADH}$ Succinate + NADPH

Abbreviations used:
NADP = Nicotinamide Adenine Dinucleotide, Oxidized Form
SSADH = Succinic Semialdehyde Dehydrogenase
NADPH = Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 25°C, pH 8.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Pyrophosphate Buffer, pH 8.6 at 25°C
(Prepare 100 ml in deionized water using Tetrapotassium Pyrophosphate, Anhydrous, Sigma Prod. No. P-8260. Adjust to pH 8.6 at 25°C with 1 M H_3PO_4.)

B. 100 mM 2-Mercaptoethanol Solution (2-ME)
(Prepare 10 ml in deionized water using 2-Mercaptoethanol, Sigma Prod. No. M-6250.)

C. 25 mM ß-Nicotinamide Adenine Dinucleotide Phosphate Solution (NADP)
(Prepare 10 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505 or dissolve the contents of one 10 mg vial of ß-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-310, in the appropriate volume of deionized water.)

D. 50 mM Succinic Semialdehyde Substrate Solution (Substrate)
(Prepare 1 ml in Reagent A using Succinic Semialdehyde, Sigma Prod. No. S-1505.)

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REAGENTS: (continued)

E. 75 mM Potassium Phosphate Buffer, with 25% (v/v) Glycerol, pH 7.2 at 25°C (Enzyme Diluent) (Prepare 25 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379 and Glycerol, Sigma Prod. No. G-9012. Adjust to pH 7.2 at 25°C using 1 M KOH.)

F. Succinic Semialdehyde Dehydrogenase Enzyme Solution (Immediately before use, prepare a solution containing 0.25 - 0.5 unit/ml of Succinic Semialdehyde Dehydrogenase in cold Reagent E.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Buffer)	23.10
Reagent B (2-ME)	0.90

Mix by swirling and equilibrate to 25°C.

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reaction Cocktail	2.40	2.40
Reagent C (NADP)	0.15	0.15
Reagent D (Substrate)	0.30	0.30

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

Reagent F (Enzyme Solution)	0.10	------
Reagent E (Enzyme Diluent)	------	0.10

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

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CALCULATIONS:

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

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADP 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 convert 1.0 µmole of succinic semialdehyde to succinate per minute with a stoichiometric reduction of 1.0 µmole of $NADP^+$ at pH 8.6 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 87 mM potassium pyrophosphate, 3 mM 2-mercaptoethanol, 1.3 mM ß-nicotinamide adenine dinucleotide phosphate, 5.0 mM succinic semialdehyde, 0.83% (v/v) glycerol, 2.5 mM potassium phosphate, and 0.025 - 0.05 unit succinic semialdehyde dehydrogenase.

NOTES:

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