

**Enzymatic Assay of SUCCINIC SEMIALDEHYDE DEHYDROGENASE  
(EC 1.2.1.16)**

**PRINCIPLE:**

Succinic Semialdehyde + NADP + H<sub>2</sub>O  $\xrightarrow{\text{SSADH}}$  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<sub>340nm</sub>, 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<sub>3</sub>PO<sub>4</sub>.)
- 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 β-Nicotinamide Adenine Dinucleotide Phosphate  
Solution (NADP)  
(Prepare 10 ml in deionized water 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 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:

	<u>Test</u>	<u>Blank</u>
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  $A_{340\text{nm}}$  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  $A_{340\text{nm}}$  for approximately 5 minutes. Obtain the  $r A_{340\text{nm}}/\text{minute}$  using the maximum linear rate for both the Test and Blank.

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**CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -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  $\mu$ mole of succinic semialdehyde to succinate per minute with a stoichiometric reduction of 1.0  $\mu$ mole of NADP<sup>+</sup> 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  $\beta$ -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.

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