Enzymatic Assay of SARCOSINE OXIDASE
(EC 1.5.3.1)

PRINCIPLE:

Sarcosine + H_2O + O_2 $\xrightarrow{\text{Sarcosine Oxidase}}$ Glycine + Formaldehyde + H_2O_2

CONDITIONS: T = 37°C, pH = 8.3, A_{410nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 60 mM Glycylglycine Buffer, pH 8.3 at 37°C
(Prepare 100 ml in deionized water using Gly-Gly, Free Base, Sigma Prod. No. G-1002. Adjust to pH 8.3 at 37°C with 1 M NaOH.)

B. 300 mM Sarcosine Solution (Sarcosine)
(Prepare 10 ml in Reagent A using Sarcosine, Free Base, Sigma Prod. No. S-9881.)

C. 500 mM Acetic Acid (CH_3COOH)
(Prepare 25 ml in deionized water using Acetic Acid, Glacial, Sigma Prod. No. A-6283.)

D. 2 M Ammonium Acetate Solution (Amm Acet)
(Prepare 40 ml in deionized water using Ammonium Acetate, Sigma Prod. No. A-7262.)

E. Isopropanol
(Use Isopropanol, Anhydrous, Sigma Stock No. 405-7.)

F. Acetylacetone
(Use Acetylacetone, Sigma Prod. No. A-3511.)

G. Color Reagent
(Prepare by adding 20 ml of Reagent D to 40 ml of Reagent E. Then add 0.15 ml of Reagent F. Mix by swirling and store overnight at 0-5°C.)

H. Sarcosine Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Sarcosine Oxidase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	0.20	0.30
Reagent B (Sarcosine)	0.20	0.20

Mix by inversion and equilibrate to 37°C. Then add:

Reagent H (Enzyme Solution)	0.10	------

Immediately mix by swirling and incubate for exactly 10 minutes at 37°C. Then add:

Reagent C (CH_3COOH)	0.50	0.50

Mix by swirling. Then add:

Reagent G (Color Reagent)	3.00	3.00

Mix by swirling and incubate at 60°C for 30 minutes. Transfer the solution to suitable cuvettes and record the A_{410nm} for both the Test and Blank in a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{410nm}\ \text{Test} - A_{410nm}\ \text{Blank})\ (4)\ (df)}{(10)(8)(0.1)}$$

4 = Total volume (in milliliters) of assay
df = Dilution factor
10 = Time of assay (in minutes) as per the Unit Definition
8 = Millimolar extinction coefficient of colored product
0.1 = Volume (in milliliters) of enzyme used

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

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

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UNIT DEFINITION:

One unit will form 1.0 µmole of formaldehyde from sarcosine per minute at pH 8.3 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 0.50 ml reaction mix, the final concentrations are 60 mM glycylglycine, 120 mM sarcosine, and 0.05 - 0.10 unit sarcosine oxidase.

REFERENCE:

Suzuki, M. (1981) *J. Biochemistry* **89**, 599-607.

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.