

**Enzymatic Assay of SARCOSINE OXIDASE
(EC 1.5.3.1)**

PRINCIPLE:

Sarcosine + O₂ + H₂O $\xrightarrow{\text{Sarcosine Oxidase}}$ Glycine + Formaldehyde + H₂O₂

2 H₂O₂ + Phenol + 4-AAP $\xrightarrow{\text{Peroxidase}}$ 4 H₂O + Quinoneimine Dye

Abbreviations:

4-AAP = 4-Aminoantipyrine

CONDITIONS: T = 37°C, pH = 8.3, A_{480nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 200 mM Tris HCl Buffer, pH 8.3 at 37°C.
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Prod. No. T-3253. Adjust to pH 8.3 at 37°C with 1 M NaOH.)
- B. 15 mM 4-Aminoantipyrine Solution (4-AAP)
(Prepare 1 ml in deionized water using 4-Aminoantipyrine Free Base, Prod. No. A-4382.)
- C. 0.2% Phenol Solution (Phenol)
(Prepare 1 ml in deionized water using Phenol, Prod. No. P-3653.)
- D. Peroxidase Enzyme Solution (POD)
(Immediately before use, prepare a solution containing 50 purpurogalin units/ml in deionized water using Prod. No. P-8250.)
- E. 1000 mM Sarcosine Solution (Sarc)
(Prepare 1 ml in deionized water using Sarcosine, Free Base, Prod. No. S-9881.)
- F. Ethanol (EtOH)
(Use Reagent Grade Ethanol.)

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REAGENTS: (continued)

- G. 10 mM Potassium Phosphate Solution, pH 7.5 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Prod. No. P-5379. Adjust to pH 7.5 at 37°C with 1 M NaOH.)
- H. Sarcosine Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 1.00 units/ml in cold Reagent G.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	0.20	0.20
Reagent A (Buffer)	0.05	0.05
Reagent B (4-AAP)	0.05	0.05
Reagent C (Phenol)	0.05	0.05
Reagent D (POD)	0.05	0.05
Reagent E (Sarc)	0.10	0.10

Mix and equilibrate to 37°C. Then add:

Reagent G (Diluent)	-----	0.01
Reagent H (Enzyme Solution)	0.01	-----

Mix and incubate at 37°C for exactly 5 min. Then add:

Reagent F (EtOH)	2.50	2.50
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Mix. Record the $A_{480\text{nm}}$ for both Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/mg Protein} = \frac{(A_{480\text{nm}} \text{ Test} - A_{480\text{nm}} \text{ Blank}) (3.01) (2)}{(17.14) (5) (\text{mg Protein/RM})}$$

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CALCULATIONS: (continued)

3.01 = Total Volume of Assay
2 = Two Hydrogen Peroxide molecules used per Molecule of
17.14 = Millimolar Extinction Coefficient of the
Quinoneimine
dye at 480 nm
5 = Time of assay in minutes
RM = Reaction Mix

UNIT DEFINITION:

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

FINAL ASSAY CONCENTRATION:

In a 0.51 ml reaction mix, the final concentrations are
49 mM Tris HCl, 1.5 mM 4-aminoantipyrine, 0.02% phenol,
2.5 purpurogallin units peroxidase, 196 mM sarcosine, 0.2
mM potassium phosphate and 0.0023 - 0.01 units sarcosine
oxidase.

NOTES:

1. All product and stock numbers, unless otherwise
indicated, are Sigma product and stock numbers.

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