Enzymatic Assay of ACYL COENZYME A OXIDASE
(EC 1.3.3.6)

PRINCIPLE:

$$\text{Palmitoyl-CoA} + O_2 \xrightarrow{\text{Acyl Coenzyme A Oxidase}} \text{2-Hexadecenoyl-CoA} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4H_2O$$

Abbreviation used:
POD = Peroxidase

CONDITIONS: T = 30°C, pH = 8.0, A_{500nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM MES Buffer, pH 8.0 at 30°C
(Prepare 200 ml in deionized water using MES Free Acid, Sigma Prod. No. M-8250. Adjust to pH 8.0 at 30°C with
1 M NaOH.)

B. 0.5% (w/v) Palmitoyl-CoA Solution (Pal-CoA)
(Prepare 10 ml in deionized water using Palmitoyl Coenzyme A, Free Acid, Sigma Prod. No. P-9276.)

C. 1.6 mM 4-Aminoantipyrine with 22 mM Phenol Solution (4-AAP)
(Prepare 100 ml in Reagent A using 4-Aminoantipyrine Free Base, Sigma Prod. No. A-4382, and Phenol, Sigma Prod. No. P-3653.)

D. 1 mM Flavin Adenine Dinucleotide Solution (FAD)
(Prepare 5 ml in Reagent A using Flavin Adenine Dinucleotide, Disodium Salt, Sigma Prod. No. F-6625. **PREPARE FRESH**.)

E. Peroxidase Enzyme Solution (POD)
(Immediately before use, prepare a solution containing 100 purpurogallin units/ml in Reagent A using Peroxidase, Sigma Prod. No. P-8250.)

REAGENTS: (continued)

F. 10% (v/v) Triton[1] X-100 Solution (X-100)
(Prepare 10 ml in deionized water using Triton[1] X-100, Sigma Stock No. X-100).

G. Acyl Coenzyme A Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.15 - 0.30 unit/ml of Acyl Coenzyme A Oxidase in cold Reagent A.

PROCEDURE:

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

Reagent A (Buffer)	13.35
Reagent C (4-AAP)	15.00
Reagent D (FAD)	0.15
Reagent E (POD)	1.50

Mix by swirling. Adjust to pH 8.0 at 30°C with 100 mM HCl or 100 mM NaOH, if necessary.

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

	Test	Blank
Reaction Cocktail	3.00	3.00
Reagent B (Pal-CoA)	0.30	0.30
Reagent F (X-100)	0.03	0.03

Mix by inversion and equilibrate to 30°C. Monitor the A_{500nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent A (Buffer)	------	0.10
Reagent G (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the increase in A_{500nm} for approximately 5 minutes. Obtain the $_{r}$ A_{500nm}/minute using the maximum linear rate for both the Test and Blank.

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{500nm}/\text{min Test} - \text{r}A_{500nm}/\text{min Blank})(2)(3.43)(df)}{(12.78)\ (0.1)}$$

2 = 2 moles H_2O_2 used per mole of dye
3.43 = Total volume (in milliliters) of assay
df = Dilution factor
12.78 = Millimolar extinction coefficient of Quinoneimine Dye
at 500 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 form 1.0 µmole of H_2O_2 and hexadecenoyl-CoA from palmitoyl-CoA per minute at pH 8.0 at 30°C in a peroxidase coupled system.

FINAL ASSAY CONCENTRATION:

In a 3.43 ml reaction mix, the final concentrations are 45 mM MES, 0.04% (w/v) palmitoyl-CoA, 0.70 mM 4-aminoantipyrine, 0.004 mM flavin adenine dinucleotide, 9.6 mM phenol, 0.09% (v/v) Triton X-100, 15 purpurogallin units peroxidase, and 0.015 - 0.030 unit acyl coenzyme A oxidase.

NOTES:

1. Triton is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.

2. Peroxidase Unit Definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 sec at pH 6.0 at 20°C.

3. Where Sigma Product or Stock numbers are specified,

equivalent reagents may be substituted.

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