



SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

Enzymatic Assay of ACYL COENZYME A OXIDASE (EC 1.3.3.6)

PRINCIPLE:

Palmitoyl-CoA + O₂ $\xrightarrow{\text{Acyl Coenzyme A Oxidase}}$ 2-Hexadecenoyl-CoA + H₂O₂

2H₂O₂ + 4-Aminoantipyrine + Phenol $\xrightarrow{\text{POD}}$ Quinoneimine dye + 4H₂O

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.)

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REAGENTS: (continued)

- F. 10% (v/v) Triton¹ X-100 Solution (X-100)
(Prepare 10 ml in deionized water using Triton¹ 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:

	<u>Test</u>	<u>Blank</u>
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 $A_{500\text{nm}}$ 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 $A_{500\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{500\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

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CALCULATIONS:

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

2 = 2 moles H₂O₂ 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 H₂O₂ 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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