Enzymatic Assay of ACYL COENZYME A OXIDASE
(EC 1.3.3.6)

PRINCIPLE:

\[
\text{Palmitoyl-CoA + O}_2 \xrightarrow{\text{Acyl Coenzyme A Oxidase}} 2\text{-Hexadecenoyl-CoA + H}_2\text{O}
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine + Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye + 4H}_2\text{O}
\]

Abbreviation used:

POD = Peroxidase

CONDITIONS: \( T = 30^\circ\text{C}, \text{pH} = 8.0, A_{500nm}, \text{Light path} = 1 \text{ cm} \)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM MES Buffer, pH 8.0 at 30\(^\circ\)C
   (Prepare 200 ml in deionized water using MES Free Acid. Adjust to pH 8.0 at 30\(^\circ\)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)

C. 1.6 mM 4-Aminoantipyrine with 22 mM Phenol Solution (4-AAP)
   (Prepare 100 ml in Reagent A using 4-Aminoantipyrine Free Base, and Phenol)

D. 1 mM Flavin Adenine Dinucleotide Solution (FAD)
   (Prepare 5 ml in Reagent A using Flavin Adenine Dinucleotide, Disodium Salt
   \text{PREPARE FRESH}.)

E. Peroxidase Enzyme Solution (POD)
   (Immediately before use, prepare a solution containing 100 purpurogallin units/ml in Reagent A using Peroxidase)
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REAGENTS: (continued)

F. 10% (v/v) Triton\textsuperscript{X-100} Solution (X-100)
(Prepare 10 ml in deionized water using Triton\textsuperscript{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:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>13.35</td>
</tr>
<tr>
<td>Reagent C (4-AAP)</td>
<td>15.00</td>
</tr>
<tr>
<td>Reagent D (FAD)</td>
<td>0.15</td>
</tr>
<tr>
<td>Reagent E (POD)</td>
<td>1.50</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Reagent B (Pal-CoA)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent F (X-100)</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>------</td>
</tr>
<tr>
<td>Reagent G (Enzyme Solution)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

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

2 = 2 moles \(H_2O_2\) used per mole of dye  
3.43 = Total volume (in milliliters) of assay  
\(\text{df}\) = Dilution factor  
12.78 = Millimolar extinction coefficient of Quinoneimine Dye  
\(r_{A_{500nm}}\) 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 hexadecenooyl-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 \textbf{OUR} 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 our quality control procedure contact our Technical Service Department.