**SIGMA QUALITY CONTROL TEST PROCEDURE**

**Enzymatic Assay of CHOLINE OXIDASE**
**(EC 1.1.3.17)**

**PRINCIPLE:**

\[
\text{Choline} + O_2 \xrightarrow{\text{Choline Oxidase}} \text{Betaine Aldehyde} + H_2O_2
\]

\[
\text{Betaine Aldehyde} + O_2 + H_2O \xrightarrow{\text{Choline Oxidase}} \text{Betaine} + H_2O_2
\]

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

Abbreviation used:

POD = Peroxidase

**CONDITIONS:**

\[ T = 37^\circ C, \text{pH} = 8.0, A_{500nm}, \text{Light path} = 1 \text{ cm} \]

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 100 mM Tris HCl Buffer, pH 8.0 at 37°C
   (Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 37°C with 1 M HCl.)

B. 2.1% (w/v) Choline Chloride Solution (Choline)
   (Prepare 100 ml in Reagent A using Choline, Chloride Salt, Sigma Prod. No. C-1879.)

C. 1% (w/v) 4-Aminoantipyrine Solution (4-AAP)
   (Prepare 2 ml in deionized water using 4-Aminoantipyrine, Free Base, Sigma Prod. No. A-4382.)

D. 1% (w/v) Phenol Solution (Phenol)
   (Prepare 5 ml in deionized water using Phenol, Sigma Prod. No. P-3653.)

E. 10 mM Tris HCl with 2.0 mM Ethylenediaminetetraacetic Acid and 134 mM Potassium Chloride Solution (Enz Dil)
   (Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS, and Potassium Chloride, Sigma Prod. No. P-4504. Adjust to pH 8.0 at 37°C with 1 M HCl.)
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REAGENTS: (continued)

F. Peroxidase Enzyme (POD)
   (Use Peroxidase, Sigma Prod. No. P-8250.)

G. Choline Oxidase Enzyme Solution (Choline Oxidase)
   (Immediately before use, prepare a solution containing 0.1 - 0.5 unit/ml of Choline Oxidase in cold Reagent E.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th>Reagent B (Choline)</th>
<th>97.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (4-AAP)</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent D (Phenol)</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent F (POD, Purpurogallin units)</td>
<td>500</td>
</tr>
</tbody>
</table>

Mix by swirling.

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>
</tbody>
</table>

Equilibrate to 37°C. Monitor the A₅₀₀ nm until constant, using a suitably thermostatted spectrophotometer. Then add:

| Reagent E (Enz Dil) | ----- | 0.05 |
| Reagent G (Choline Oxidase) | 0.05 | ----- |

Immediately mix by inversion and record the increase in A₅₀₀ nm for approximately 5 minutes. Obtain the ΔA₅₀₀ nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{500\text{nm}}/\text{min Test} - \Delta A_{500\text{nm}}/\text{min Blank})(3.05)(df)}{(12)(0.5)(0.05)}
\]

3.05 = Volume (in milliliters) of assay
df = Dilution factor
12 = Millimolar extinction coefficient of Quinoneimine Dye at 500 nm under the conditions of the assay
0.5 = \( \mu \text{mole of Quinoneimine Dye formed per } \mu \text{mole of } \text{H}_2\text{O}_2 \)
0.05 = Volume (in milliliter) of choline oxidase used in assay
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CALCULATIONS:

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

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

UNIT DEFINITION:

One unit will form 1.0 \( \mu \text{mole} \) of \( \text{H}_2\text{O}_2 \) from the oxidation of 1 \( \mu \text{mole} \) of choline to betaine aldehyde per minute at pH 8.0 at 37\(^\circ\)C.

FINAL ASSAY CONCENTRATION:

In a 3.05 ml reaction mix, the final concentrations are 96 mM Tris, 2.0\% (w/v) choline, 0.01\% (w/v) 4-aminoantipyrine, 0.02\% (w/v) phenol, 15 units peroxidase, 0.03 mM ethylenediaminetetraacetic acid, 2 mM potassium chloride and 0.005 - 0.025 unit choline oxidase.

REFERENCES:


NOTES:

1. The millimolar extinction coefficient is described in Keesey, J. (1982).

2. This assay is based on the cited references.

3. Peroxidase Unit Definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20\(^\circ\)C.

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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