Enzymatic Assay of CHOLINE OXIDASE
(EC 1.1.3.17)

PRINCIPLE:

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

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

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

Abbreviation used:
POD = Peroxidase

CONDITIONS: T = 37°C, pH = 8.0, A_{500nm}, Light path = 1 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:

Reagent B (Choline)	97.00
Reagent C (4-AAP)	1.00
Reagent D (Phenol)	2.00
Reagent F (POD, Purpurogallin units)	500

Mix by swirling.

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

	Test	Blank
Reaction Cocktail	3.00	3.00

Equilibrate to 37°C. Monitor the A_{500nm} 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 A_{500nm} for approximately 5 minutes. Obtain the ΔA_{500nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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[1]
0.5 = µmole of Quinoneimine Dye formed per µmole of H_2O_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 µmole of H_2O_2 from the oxidation of 1 µmole of choline to betaine aldehyde per minute at pH 8.0 at 37°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:

Okabe, H., Sagesaka, K., Nakajima, N., and Noma, A. (1977) *Clinica Chimica Acta* **80**, 87-94

Keesey, J. (1987) *Biochemica Information*, 1st ed., pp 19-20, Boehringer Mannheim Biochemicals, IN

NOTES:

1. The millimolar extinction coefficient is described in Kessey, 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°C.

4. 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.