

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

**PRINCIPLE:**

Choline + O<sub>2</sub>  $\xrightarrow{\text{Choline Oxidase}}$  Betaine Aldehyde + H<sub>2</sub>O<sub>2</sub>

Betaine Aldehyde + O<sub>2</sub> + H<sub>2</sub>O  $\xrightarrow{\text{Choline Oxidase}}$  Betaine + H<sub>2</sub>O<sub>2</sub>

2H<sub>2</sub>O<sub>2</sub> + 4-Aminoantipyrine + Phenol  $\xrightarrow{\text{POD}}$  Quinoneimine dye + 4 H<sub>2</sub>O

Abbreviation used:  
POD = Peroxidase

**CONDITIONS:** T = 37°C, pH = 8.0, A<sub>500nm</sub>, 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:

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

**CALCULATIONS:**

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

- 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<sup>1</sup>
- 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°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.**