

**Enzymatic Assay of CHOLESTEROL OXIDASE
(EC 1.1.3.6)**

PRINCIPLE:

Cholesterol + O₂ $\xrightarrow{\text{Cholesterol Oxidase}}$ 4-Cholesten-3-one + H₂O₂

CONDITIONS: T = 37°C, pH = 5.0, A_{243nm}, Light path = 1 cm

METHOD: Stopped Spectrophotometric Rate Determination

REAGENTS:

- A. 0.5% (w/v) Cholesterol Substrate Solution
(Prepare 10 ml in Reagent B using Cholesterol, Sigma Prod. No. C-8503.)
- B. Ethyl Alcohol (EtOH)
(Use 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Corp.)
- C. 500 mM Sodium Acetate Buffer, pH 5.0 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.0 at 37°C with 1 M HCl.)
- D. 10 mM Sodium Phosphate Solution, pH 7.3 at 37°C (Enz Dil)
(Prepare 50 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.3 at 37°C with 1 M NaOH.)
- E. Cholesterol Oxidase Enzyme Solution
(Immediately before use, prepare a solution 0.003 - 0.009 unit/ml of Cholesterol Oxidase in Reagent C.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers.

	<u>Test</u>	<u>Blank</u>
Reagent A (Substrate Solution)	0.10	0.10
Reagent C (Buffer)	0.40	0.40

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 37°C. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent E (Enzyme Solution)	0.50	-----

Immediately mix by inversion and incubate at 37°C for exactly 30 minutes. Then add:

Reagent B (EtOH)	3.00	3.00
Reagent E (Enzyme Solution)	-----	0.50

Mix by inversion and transfer to suitable cuvettes. Record the A_{243nm} for both the Test and Blank in a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{243nm} \text{ Test} - A_{243nm} \text{ Blank})(4)(df)}{(30)(18)(0.5)}$$

4 = Volume (in milliliters) of the stopped reaction

df = Dilution factor

30 = Time (in minutes) of the assay as per the Unit Definition

18 = Millimolar extinction coefficient of 4-cholesten-3-one

0.5 = Volume (in milliliter) 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 convert 1.0 μ mole of cholesterol to 4-cholesten-3-one per minute at pH 5.0 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 1.0 ml reaction mix, the final concentrations are 0.05% (w/v) cholesterol, 10% (v/v) ethyl alcohol, 450 mM sodium acetate, and 0.0015 - 0.0045 unit cholesterol oxidase.

REFERENCE:

Richmond, W. (1973) *Clinical Chemistry* **19**, 1350-1356

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.