

**Enzymatic Assay of CHOLESTEROL ESTERASE
(EC 3.1.1.13)**

PRINCIPLE:

Cholesterol Oleate $\xrightarrow{\text{Cholesterol Esterase}}$ Cholesterol + Oleic Acid

Cholesterol + O₂ + H₂O $\xrightarrow{\text{Cholesterol Oxidase}}$ H₂O₂ + Cholestenone

2H₂O₂ + 4-AAP + Phenol $\xrightarrow{\text{Peroxidase}}$ 4H₂O + Quinoneimine Dye

Abbreviations:

4-AAP = 4-Aminoantipyrine

CONDITIONS: T = 37°C, pH = 7.0, A_{500nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 400 mM Potassium Phosphate Buffer, pH 7.0 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379 and Potassium Phosphate Dibasic, Trihydrate, Prod. No. P-5504. Adjust to pH 7.0 at 37°C with 1 M NaOH.)
- B. 0.9% (w/v) Sodium Chloride Solution
(Prepare 25 ml in deionized water using Sodium Chloride, Prod. No. S-9625.)
- C. 8.6 mM Cholesteryl Oleate Solution (Chol-Oleate)
(Prepare by first dissolving 56.0 mg of Cholesteryl Oleate, Prod. No. C-9253, in 1 ml of Polyoxyethylene Ether, 9 Lauryl Ether, Prod. No. P-9641. While stirring, add 9 ml of hot Reagent B. Store at room temperature.)
- D. 15% (w/v) Taurocholic Acid Solution (Tauro)
(Prepare 10 ml in deionized water using Taurocholic Acid, Sodium Salt, Prod. No. T-0750.)

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REAGENTS: (continued)

- E. 1.75% (w/v) 4-Aminoantipyrine Solution (4-AAP)
(Prepare 1 ml in deionized water using 4-Aminoantipyrine, Free Base, Prod. No. A-4382.)
- F. 6% (w/v) Phenol Solution
(Prepare 10 ml in deionized water using Phenol, Prod. No. P-4161.)
- G. Cholesterol Oxidase Enzyme Solution (Chol Oxid)
(Immediately before use, prepare a solution containing 20 - 30 units/ml of Cholesterol Oxidase, Prod. No. C-1512, in cold Reagent A.)
- H. Peroxidase Enzyme Solution (POD)
(Immediately before use, prepare a solution containing 5 mg/ml of Peroxidase Type II from Horseradish, Prod. No. P-8250 in cold deionized water.)
- I. Cholesterol Esterase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Cholesterol Esterase in cold Reagent A.)

PROCEDURE:

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

	<u>Test</u>	
Reagent A (Buffer)	31.80	
Reagent C (Chol-Oleate)		7.50
Reagent D (Tauro)	1.50	
Reagent E (4-AAP)	0.75	
Reagent F (Phenol)	1.50	
Reagent G (Chol Oxid)	0.30	
Reagent H (POD)	0.15	

Mix by inversion and equilibrate to 37° C.

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PROCEDURE: (continued)

Pipette (in milliliters) the following reagents into a suitable container:

	<u>Test</u>	<u>Test</u>
Reaction Cocktail	2.90	2.90
Reagent I (Cholesterol Esterase)	0.10	----
Reagent A (Buffer)	----	0.10

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

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{\Delta A_{500\text{nm}}/\text{min Test} - \Delta A_{500\text{nm}}/\text{min Blank}}{(0.5) (13.78) (\text{mg enzyme/ml RM})}$$

13.78 = Millimolar extinction coefficient of Quinoneimine Dye

at 500 nm under the assay conditions

RM = Reaction Mix

0.5 = Conversion factor based on one mole of H_2O_2 produces half a mole of Quinoneimine Dye.

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of cholesteryl oleate to cholesterol and oleic acid per minute at pH 7.0 at 37°C in the presence of taurocholate.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 299 mM potassium phosphate, 0.50% (w/v) taurocholic acid, 0.05 mg peroxidase, 1.4 mM cholesteryl oleate, 1.7% (w/v) polyoxyethylene 9 lauryl ether, 0.14% (w/v) sodium chloride, 0.2% (w/v) phenol, 0.4-0.6 unit cholesterol oxidase and 0.025 - 0.05 unit cholesterol esterase.

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NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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