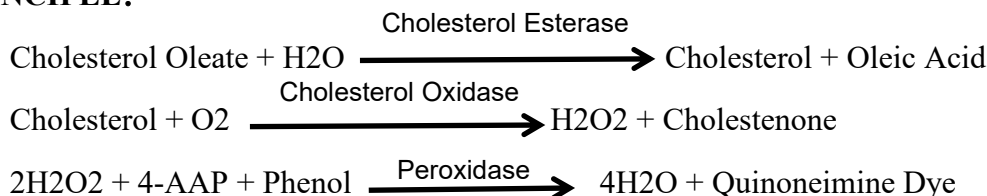




胆固醇酯酶

PRINCIPLE:



Abbreviation:

4-AAP = 4-Aminoantipyrine

CONDITIONS:

T = 37°C, pH = 7.0, A500nm, 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, Adjust to pH 7.0 at 37°C with 1 M KOH.)

B. 0.9% (w/v) Sodium Chloride Solution

(Prepare 25 ml in deionized water using Sodium Chloride.)

C. 8.6 mM Cholesteryl Oleate Solution (Chol-Oleate)

(Prepare 10 ml by first dissolving the Cholesteryl Oleate, in 1 ml of Polyoxyethylene, 9 Lauryl Ether. Stir gently, with heat, until the solution is clear and colorless. Then add 9 ml of hot Reagent B and continue for 5 minutes. Allow the solution to return to ambient temperature prior to use. The solution clears upon cooling.)

D. 15% (w/v) Taurocholic Acid Solution (Tauro)

(Prepare 10 ml in deionized water using Taurocholic Acid, Sodium Salt.)

E. 15% (w/v) Cholic Acid Solution (Chol)



(Prepare 10 ml in deionized water using Cholic Acid, Sodium Salt.)

F. 1.76% (w/v) 4-Aminoantipyrine Solution (4-AAP)

(Prepare 1 ml in deionized water using 4-Aminoantipyrine, Free Base.)

G. 5% (w/v) Phenol Solution (Phenol)

(Prepare 10 ml in deionized water using Phenol.)

H. Cholesterol Oxidase Enzyme Solution (Chol Oxid)

(Immediately before use, prepare a solution containing 20 - 30 units/ml of Cholesterol Oxidase, in cold Reagent A.)

I. Peroxidase Enzyme Solution (POD)

(Immediately before use, prepare a solution containing 40 - 60 units/ml of Peroxidase Type II from Horseradish in cold deionized water.)

J. Cholesterol Esterase Enzyme Solution

(Immediately before use, prepare a solution containing 0.25 - 0.85 unit/ml of Cholesterol Esterase in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes1:

	Test	Blank
Reagent A (Buffer)	2.10	2.10
Reagent D (Tauro)	0.05	0.05
Reagent E (Chol)	0.05	0.05
Reagent I (POD)	0.10	0.10
Reagent C (Chol-Oleate)	0.50	0.50
Reagent G (Phenol)	0.05	0.05

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

Reagent F (4-AAP)	0.05	0.05
Reagent H (Chol Oxid)	0.05	0.05

Mix by inversion and obtain the baseline at 500 nm. After approximately 5 minutes add:



	Test	Blank
Reagent J (Cholesterol Esterase)	0.05	-----
Reagent A (Buffer)	-----	0.05

Immediately mix by inversion and record the increase in A500nm for approximately 10 minutes.² Obtain the $\Delta A_{500nm}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{500nm}/\text{min Test} - \Delta A_{500nm}/\text{min Blank})(3)(df)}{(0.5)(13.78)(0.05)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

0.5 = Conversion factor based on one mole of H₂O₂ produces half a mole of Quinoneimine Dye

0.05 = Volume (in milliliters) of enzyme used

13.78 = Millimolar extinction coefficient of Quinoneimine Dye at 500 nm under the assay conditions

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

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

UNIT DEFINITION:

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

FINAL ASSAY CONCENTRATION:

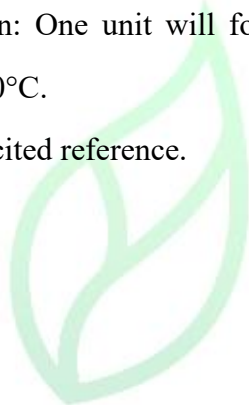
In a 3.00 ml reaction mix, the final concentrations are 287 mM potassium phosphate, 0.25% (w/v) taurocholic acid, 0.25% (w/v) cholic acid, 4 - 6 units peroxidase, 1.4 mM cholesteryl oleate, 1.7% (v/v) polyoxyethylene 9 lauryl ether,



0.14% (w/v) sodium chloride, 0.083% (w/v) phenol, 0.03% (w/v) 4-aminoantipyrine,
1 - 1.5 units cholesterol oxidase and 0.013 - 0.043 unit cholesterol esterase.

NOTES:

1. Add the reagents in the order written.
2. The fastest rate is usually between 4-8 minutes after addition of the Cholesterol Esterase.
3. Cholesterol Oxidase Unit Definition: One unit will convert 1.0umole of cholesterol to 4-cholesten-3-one per minute at pH 7.5 at 25°C.
4. Peroxidase Unit Definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 sec at pH 6.0 at 20°C.
5. This assay is based on the cited reference.



源叶