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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of CHOLESTEROL OXIDASE[1]
(EC 1.1.3.6)

PRINCIPLE:

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ H_2O_2 + 4-Cholesten-3-one

H_2O_2 + o-Dianisidine (reduced) $\xrightarrow{\text{Peroxidase}}$ 2 H_2O + o-Dianisidine (oxidized)

CONDITIONS: T = 25°C, pH = 7.5, A_{500nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer, pH 7.5 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 25°C with 1 M KOH.)

B. 1% (w/v) o-Dianisidine Solution (ODA)
(Dissolve the contents of a 50 mg vial of o-Dianisidine Dihydrochloride, Sigma Stock No. 510-50, in 5 ml of deionized water **or** prepare 5 ml in deionized water using o-Dianisidine Dihydrochloride, Sigma Prod. No. D-3252. **PREPARE FRESH**.)

C. 0.5% (w/v) Cholesterol with 10% (v/v) Triton[2] X-100 Solution (Chol)
(Prepare by initially dissolving the Cholesterol, Sigma Prod. No. C-8503, in 10 ml of Triton[2] X-100. Heat until the solution clarifies. Then add 90 ml of deionized water. Stir well. Store the solution at 4°C.)[3]

D. Peroxidase Enzyme Solution (POD)
(Immediately before use, prepare a solution containing 100 pyrogallol units/ml in deionized water using Peroxidase from Horseradish, Sigma Prod. No. P-8250.)

E. Cholesterol Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Cholesterol Oxidase in cold Reagent A.)

PROCEDURE:

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

Reagent A (Buffer)	40.0
Reagent B (ODA)	0.50

Mix thoroughly by swirling and adjust to pH 7.5 at 25°C with 100 mM HCl or 100 mM KOH, if necessary. Then add Reagent A to a final volume of 50 ml. Mix by swirling thoroughly and oxygenate for approximately 10 minutes immediately before use.

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

	Test	Blank
Reaction Cocktail	2.70	2.70
Reagent C (Chol)	0.10	0.10
Reagent D (POD)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{500nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent A (Buffer)	------	0.10
Reagent E (Enzyme Solution)	0.10	------

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:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{500nm}/\text{min Test} - \Delta A_{500nm}/\text{min Blank})(3)(df)}{(7.5)\,(0.1)}$$

3 = Volume (in milliliters) of assay
df = Dilution factor
7.5 = Millimolar extinction coefficient of o-Dianisidine (Oxidized) at 500 nm
0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of cholesterol to 4-cholesten-3-one per minute at pH 7.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 38 mM potassium phosphate, 0.009% (w/v) o-dianisidine, 0.017% (w/v) cholesterol, 0.33% (v/v) Triton X-100, 10 units peroxidase and 0.01 - 0.02 unit cholesterol oxidase.

REFERENCE:

Masurekar, P.S. and Goodhue, C.T. (1978) United States Patent No. 4,093,517, Eastman Kodak Company, Rochester, NY

NOTES:

1. This assay is not to be used to assay Cholesterol Oxidase from Schizophyllum commune, Sigma Prod. No. C-7274 and Cholesterol Oxidase from Brevibacterium sp., Sigma Prod. No. C-8153.

2. Triton is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.

3. The solution can still be used if it becomes cloudy upon addition of the water.

4. Peroxidase Unit Definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20°C.

5. This assay is based on the cited reference.

6. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.