Cholesterol Esterase (COE) from Pseudomonas sp.

C1492993

Storage Store at -20℃ long term (12 months). Upon reconstitution, it is recommended to aliquot. Avoid freeze/thaw cycle.

Shipping Ship under low temperature. Upon receipt, please promptly store under the specified storage conditions.

Introduction:

Cholesterol Esterase from Pseudomonas sp. is an enzyme that hydrolyzes cholesterol ester to cholesterol and free fatty acid in the intestinal lumen. Cholesterol synthesized in the acinar cells and is stored in zymogen granules. Cholesterol esterase is also known as bile salt-stimulated lipase and carboxy ester lipasea, acts function for acceleration of cholesterol absorption.

Cholesterol ester + H_2O → Cholesterol + Fatty acid.

Preparation and Specification

Appearance	White to pale brownish amorphous powder, lyophilized
Activity	≥100U/mg powder

Properties

Molecular weight	30 kDa (SDS-PAGE)
Isoelectric point	pH 5.28 (estimated from amino acid sequence)
Optimum pH	6.5
pH stability	6.0-11.0 (37℃, 60min, in 0.1% BSA)
Optimum temperature	35-40℃ (Phosphate Buffer)
Thermal stability	Stable at 45℃ and below (pH 8.0, 30min)
Storage stability	At least one year at -20℃.
Activator	Adekatol TN-100, Adekatol PC-8, Adelatol SO-120, Adekatol SO-135, Triton X-100

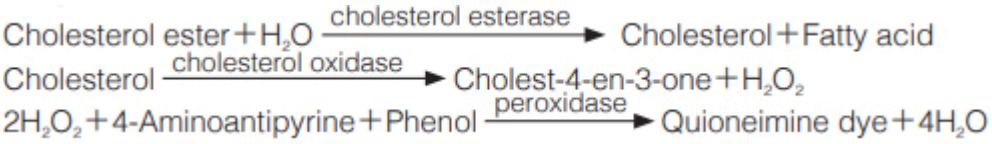
Applications

This enzyme is useful for enzymatic determination of total cholesterol, HDL-C, and LDL-C coupled with cholesterol oxidase.

ASSAY

Principle:

The assay is based on the increase in absorbance at 493 nm as the formation of quinoneimine dye proceeds in the following reactions:



Unit definition:

One unit is defined as the amount of enzyme which liberates 1 μmole of cholesterol per minute at 37℃ under the conditions specified in the assay procedure.

Reagents:

1. Reaction mixture

Buffer	Volume
0.2M KH_2PO_4-NaOH buffer, pH 6.8	0.60 ml
0.35% (W/V) 4-AA solution	0.30 ml
0.2% (W/V) Phenol solution	0.30 ml
100 U/ml HRP (Horseradish Peroxidase) solution①	0.30 ml
3% (W/V) Triton X-100 solution	0.30 ml
0.2 U/ml CHO (Cholesterol Oxidase) solution②	0.60 ml
Substrate solution③	0.30 ml
Distilled water	0.30 ml

① 100 U/ml HRP solution: Dissolve 1000 U (PPU) of HRP with 10ml of distilled water.

② 0.2 U/ml CHO solution: Dissolve 2 U of CHO with CHO dilution buffer※

※ CHO dilution buffer: 0.1M KH_2PO_4-Na_2HPO_4 buffer, pH 7.0 containing 0.05% (W/V) Triton X-100.

③ Substrate solution: Calf serum.

2. Enzyme dilution buffer: 10mM KH_2PO_4-NaOH buffer pH 7.5 containing 0.1% (W/V) bovine serum albumin (BSA).
3. Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20ml. Dilute it with enzyme dilution buffer to adjust the concentration to within 0.3-0.5 U/ml.

Procedure

1. Pipette accurately 3.0ml of reaction mixture into a small test tube and preincubate it at 37℃.
2. After 10min, add 50μl of enzyme solution and mix to start the reaction at 37℃.

※ In the case of a test blank, add 50μl of enzyme dilution buffer in place of enzyme solution.

3. After starting the reaction, measure the rate of increase per minute in absorbance at 493 nm. The rate must be measured within the linear portion of the absorbance curve.

Absorbance sample: As/min

Blank: Ab/min

△A/min = (As/min - Ab/min) ≤ 0.050 Abs/min

Calculation

$$\text{Activity(U/mg of powder)} = \frac{\triangle \text{A/min}}{12.0 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{\text{x}}$$

12.0 : millimolar extinction coefficient of quinoneimine dye at 493 nm(cm^2/μmole)

1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produce 1 mole of quinoneimine dye

3.05 : final volume(ml)

0.05 : volume of enzyme solution(ml)

X : concentration of the sample in enzyme solution(mg/ml)