Cholesterol Esterase (CHE-XE)

from recombinant E. coli

Steryl-ester acylhydrolase, EC 3.1.1.13

Cholesterol ester + H₂O → Cholesterol + Fatty acid

SPECIFICATION

Appearance: light yellow lyophilizate
Activity: ≥5 U/mg lyophilizate
Stabilizer: sucrose
Storage: at −20°C

PROPERTIES

Molecular weight: ca. 54 kDa (gel filtration)
Structure: monomer of 54 kDa (SDS-PAGE)
Michaelis constant: 1.9×10⁻⁵ M (cholesterol linoleate)

pH Optimum: 5.5–7.0 (Fig. 1)
pH Stability: 5.0–10.0 (Fig. 2)
Optimum temperature: 40°C (Fig. 3)
Thermal stability: below 50°C (Fig. 4)

Specificity: cholesterol linoleate (100), cholesterol acetate (2)
cholesterol oleate (98), cholesterol palmitate (74)
cholesterol stearate (68), cholesterol arachidonate (46)
ASSAY PROCEDURE

Principle

\[ \text{Cholesterol esterase} \quad \text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{cholesterol esterase}} \text{Cholesterol} + \text{Fatty acid} \]

\[ \text{Cholesterol oxidase} \quad \text{Cholesterol} + \text{O}_2 \xrightarrow{\text{cholesterol oxidase}} \text{Cholest-4-en-3-one} + \text{H}_2\text{O}_2 \]

\[ 2 \text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4\text{H}_2\text{O} \]

The appearance of quinoneimine dye is measured spectrophotometrically at 500 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 \( \mu \text{mol} \) of cholesterol per min at 37°C and pH 6.0 under the conditions described below.

Reagents

A. MES-NaOH buffer, 0.2 M; pH 6.0: dissolve 8.53 g of MES in 180 ml of distilled water, adjust to pH 6.0 with 2 N NaOH solution and dilute with distilled water to 200 ml.

B. Cholesterol linoleate solution, 0.6 mM: dissolve 39 mg of cholesterol linoleate in 2.0 ml of isopropyl alcohol with heating, mix with 80 ml of hot 1.0% Triton X-100 solution, keep on a water bath (74°C) for about 30 min until the solution turns clear, cool down to room temperature in running water, dissolve 600 mg of sodium cholate and dilute with 1.0% Triton X-100 solution to 100 ml.

C. 4-Aminoantipyrine (4-AA) solution, 1.76%: 1.76 g of 4-AA/100 ml of distilled water.

D. Phenol solution, 6.0%: 6.0 g of phenol/100 ml of distilled water.

E. Peroxidase (POD) solution, 150 U/ml: 75 mg of POD (200 guaiacol U/mg)/100 ml of MES-NaOH buffer (Reagent A).

F. Cholesterol oxidase (CHO-CE) solution: 200 U/ml of MES-NaOH buffer (Reagent A).

G. Enzyme dilution buffer: 50 mM potassium phosphate buffer, pH 7.5, containing 0.2% bovine serum albumin and 2 mM MgCl₂.

H. Working solution: mix the following reagents. (Prepare immediately before measurement)

| 7.5 ml | MES-NaOH buffer (Reagent A) |
| 5.0 ml | Cholesterol linoleate solution (Reagent B) |
| 0.25 ml | 4-AA solution (Reagent C) |
| 0.50 ml | Phenol solution (Reagent D) |
| 0.50 ml | POD solution (Reagent E) |

Sample: dissolve the enzyme preparation to a volume activity of 0.08–0.20 U/ml with ice-cold enzyme dilution buffer (Reagent G) immediately before measurement.

Procedure

1. Pipette 2.2 ml of working solution (Reagent H) into a cuvette (light path: 1 cm).
2. Equilibrate at 37°C for about 5 min.
3. Add 0.08 ml of CHO-CE solution (Reagent F) and equilibrate at 37°C for about 2 min.
4. Add 0.08 ml of sample and mix.
5. Record the increase of absorbance at 500 nm in a spectrophotometer thermostated at 37°C, and calculate the \( \Delta A \) per min using the linear portion of the curve (\( \Delta A_t \)).

The blank solution is prepared by adding enzyme dilution buffer (Reagent G) instead of sample (\( \Delta A_b \)).
Calculation

Activity can be calculated by using the following formula:

\[
\text{Volume activity (U/ml)} = \frac{(A_b - A_o) \times 2.36 \text{ (ml)} \times df}{13.8 \times 1/2 \times 0.08 \text{ (ml)}} = \Delta A \times 4.28 \times df
\]

\[
\text{Weight activity (U/mg)} = (\text{U/ml}) \times 1/C
\]

13.8 : Millimolar extinction coefficient of quinoneimine dye under the assay conditions (cm^2/\mu mol)
1/2 : Factor based on the fact that 1 mol of hydrogen peroxide produces 1/2 mol of quinoneimine dye
\(df\) : Dilution factor
\(C\) : Content of cholesterol esterase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of cholesterol in clinical analysis.

\[
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\]

REFERENCES

EXPERIMENTAL DATA

Fig. 1 pH Optimum

![Graph showing pH optimum with different buffers.]

- ○: 0.1 M phosphate buffer
- ●: 0.1 M Tris-HCl buffer
- □: 0.1 M glycine-NaOH buffer

Buffer: 0.1 M phosphate buffer, pH 6.0

Fig. 2 pH Stability

![Graph showing pH stability with different buffers.]

- ○: 50 mM Tris-HCl buffer
- ▲: 50 mM acetate buffer
- ●: 50 mM phosphate buffer
- □: 50 mM citrate buffer
- △: 50 mM glycine-NaOH buffer

Treatment: 25°C, 17 h

Fig. 3 Optimum temperature

![Graph showing optimum temperature.]

Buffer: 0.1 M phosphate buffer, pH 6.0

Fig. 4 Thermal stability

![Graph showing thermal stability.]

Treatment: 50 mM HEPES-NaOH buffer, pH 7.0, 15 min