ALPHA DIAGNOSTIC INTERNATIONAL

Product Data Sheet

Cholesterol Esterase (Candida Rugosa, Fungus)

Cat# CES-03 Cholesterol esterase (25-100 U/mg), Candida Rugosa **Size:** 10,000 U

Cholesterol esterase (CE; EC #3.1.1.13) is also known as cholesterol ester hydrolase. This enzyme catalyzes the following reaction:

CE
Sterol Ester --------> Sterol + Fatty Acid

Cholesterol esterase activity has been demonstrated in pancreas, intestine, liver and kidney. The enzyme is inactivated by proteolytic enzymes but stabilized by proteolytic enzyme inhibitors and by bile salts. CE from rat pancreas has a molecular weight of 65,000-69,000. In the presence of bile salts, it aggregates to a hexamer which is possibly the active form of the enzyme

Cholesterol esterase is widely used in clinical medicine for determination of serum cholesterol. The choice of solvent will depend on the intended application. The powdered enzymes are soluble water or 0.1 M phosphate buffer, pH 6 (10 mg/ml).

Form and Storage

The powdered enzyme should be stored in the freezer (−20°C). If properly stored, these products have a shelf life of at least two years.

Purity/Specificity

Purified from Candida Rugosa (fungus). ~90% protein..

Unit Definition: That amount of enzyme which causes the production of one micromole of cholesterol per minute at 37°C under conditions described in the assay procedure.
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Activity (units per mg solid): >35 (25-100 U/mg). Lot specific acivity is provided on the vial.

Solubility: Soluble in distilled water or phopshate buffer

Assay Methods

Cholesterol esterase (CE) activity (please inquire with Calzyme for the assay method for cholesterol esterase from Candida rugosa) is assayed in a coupled reaction sequence where the free cholesterol formed by CE is oxidized by cholesterol oxidase (ChO) to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide. Finally, in the presence of peroxidase, the H2O2 oxidatively couples with 4-aminoantipyrine to produce a chromogen which has an absorbance maximum at 500 nm (Allain, C.C.; Poon, L.S.; Chan, C.S.G.; Richmond, W. and P.C. Fu, Clin Chem., 20, 470, 1974).

Reagents

1. 0.1 M Potassium phosphate buffer, pH 6.7.
2. Combined Reagent - 100 ml. Prepare by dissolving the following in 100 ml 0.1M potassium phosphate buffer, pH 6.7. Prepare fresh immediately prior to assay

   Sodium cholate 129 mg
   4-aminoantipyrine 16 mg
   Phenol 132 mg
   Polyethylene glycol-6000 102 mg
   Peroxidase 200 Units
   Cholesterol oxidase 25 Units
3. Substrate - Serachol (General Diagnostics) Specs: >375 mg/dl esters.
4. Cholesterol esterase (CE) solution (0.1-0.2 U/ml). Dissolve 10 mg of enzyme in 10 ml 0.1 M phosphate buffer. Dilute further, in the same buffer, to yield a final concentration of 0.1-0.2 U/ml. Must be prepared fresh immediately prior to assay.

Procedure

1. Set spectrophotometer (equipped with a strip chart recorder and temperature control) at 500 nm and 37°C.
2. Into a cuvette pipette the following:
3. Combined Reagent 3.00 ml Substrate 0.05 ml
4. Incubate cuvette in spectrophotometer at 37°C for 10 min. to attain temperature equilibration.
5. Record blank rate at 500 nm.
6. Add 0.1 ml enzyme solution to the cuvette. Mix and record the increase in absorbance at 500 nm for 8-10 min.
7. Calculate the ΔE500 nm/min from the linear portion of the curve.

References

(Hyun, J., Steinberg, M., Treadwell, C.R., and G.V. Vahouny, Biochem. Biophys. Res. Comm., 44, 819, 1971).

Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and P.C. Fu, Clin Chem, 20, 470, 1974).

For in vitro research use only

CES-03 160823SV

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