

Colipase, Enzyme Activity

Catalog	Unit
TBP0063-1MG	1 mg
TBP0063-5MG	5 mg

Product Details

Form: Freeze-dried powder

Solubility: Water or dilute buffer

Stability: Store at -20° C (-4° F)

Activity: 4000-12000 U/mg solid

Protein: 95%

Contaminants: Free of lipase

Unit Definition

The amount of enzyme causing the release of one micromole of fatty acid from tributyrin per minute at 25°C.

Applications

Pancreatic lipase when prepared essentially free of co-lipase is strongly inhibited by conjugated bile salts at or above their critical micell concentration. Addition of co-lipase restores activity to the bile-salt-inhibited lipase and gelfiltration experiments indicate that co-lipase in bile salt solution makes a dimer which forms a 1:1 complex with lipase. Lipase and co-lipase interact as a stoichiometrical relationship and classified co-lipase interact as a co-enzyme for lipase. The co-lipase is a single chain polypeptide with a molecular weight approximately 11,000 with 5 disulfide bridges and isoelectric point 5.0.

Reagents

1. Tributyrin (reagent grade).
2. NaOH, 0.2 M.
3. Lipase (50 Units).
4. Reaction mixture. Dissolve 0.24 g Tris, 2.09 g Sodium tauro-deoxycholate, 0.11 g CaCl₂ and 8.76 g NaCl in 800 ml of distilled H₂O. Adjust pH to 6.5 with HCl. Bring volume to 1000 ml with distilled H₂O.

Procedure

1. Into the reaction vessel of an automatic titrator pipette 15 ml of the reaction mixture and 0.5 ml of Tributyrin at 25°C.
2. Add 50 potentiometric units of lipase to the reaction vessel.
3. Maintaining the pH at 6.5 (and temperature at 22-24°C), titrate the mixture with 0.2 M NaOH over a 5 min period.
4. Calculate the blank rate.
5. To the reaction vessel add 75 µl of a colipase sample that has been diluted 1:10 with distilled H₂O.
6. Titrate the reaction for 5 minutes.
7. Calculate the rate of reaction. (if the net rate is 0.040-0.095 ml/min., continue with the calculation. If the rate is outside this range, adjust the concentration of colipase and reanalyze).

Calculation

$$\text{Activity (U/mg)} = \frac{\left[\frac{\text{ml NaOH}_{\text{sample}}}{\text{min}} - \frac{\text{ml NaOH}_{\text{blank}}}{\text{min}} \right] (\text{mM NaOH})(\text{Enz. Diln.})}{\text{sample size (ml)}}$$

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