Tribioscience

alpha-Amylase, Enzyme Activity

Catalog	Unit
TBP0054-500KU	500000 U
TBP0054-2500KU	2500000 U

Product Details

Form: Freeze-dried powder

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Activity: 50 U/mg protein

Protein: 90%

Unit Definition

The amount of enzyme which liberates, from soluble starch, one micromole of maltose per minute at 25° C, pH 6.9.

Assay Method

The reducing groups liberated from starch hydrolysis reduce 3,5-dinitrosalicylic acid, resulting in formation of a colored product which can be measured spectrophotometrically at 540 nm.

Applications

Alpha amylase (EC 3.2.1.1) is able to catalyze the hydrolysis of starch (amylose and amylopectin) to a significant extent resulting in the release of maltose. The enzyme is found in nearly all plants, animals and microorganisms. The enzymes from various sources exhibit marked differences in physical, chemical and catalytic properties. Bacterial amylases and those from porcine pancreas and human saliva have been extensively studied. Alpha amylase from porcine pancreas has a molecular weight of about 50,000 and it exhibits optimum catalytic activity at pH 7.0. The E 1%/280 is 26.0.

Alpha amylase is widely used in the food industry as a clarifying agent. Determination of serum alpha amylase activity, for the diagnosis of acute pancreatitis, is a widely used procedure in clinical medicine. Additionally, it has been reported that elevated alpha amylase activity in serum also occurs in mumps, renal disease and abdominal disorders such as cholecystitis.

Reagents

1. 0.02 M Sodium phosphate buffer, pH 6.9 containing 0.006 M sodium chloride.
2. 2.0 M Sodium hydroxide.
3. Dinitrosalicylic acid color reagent. Dissolve 1.0 g 3,5-dinitrosalicylic acid in 20 ml 2 M NaOH. Add slowly, 30.0 g sodium potassium tartrate tetrahydrate. Dilute to a final volume of 100 ml with distilled water. Store in a tightly sealed container and protected from CO2. Stable for 2 weeks.
4. 1% Starch. Dissolve 1.0 g soluble starch in 100 ml 0.02 M sodium phosphate buffer, pH 6.9, containing 0.006 M NaCl. Bring to a gentle boil to dissolve. Cool and make volume up to 100 ml, with distilled water, if necessary. Incubate at 25° C for 5 minutes prior to assay.
5. Enzyme (a-amylase) solution. Dilute to a concentration of 1-10 µg/ml. Prepare at least 3 different concentrations within this range. Must be prepared fresh immediately prior to assay.

Calculation

$$\text{Activity (U/mg)} = \frac{\text{(micromoles maltose liberated)}}{\text{(mg Enz. used in sample)(3 min.)}}$$

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