

## alpha-Amylase Inhibitor

---

Catalog	Unit
TBI5637-1MG	1 mg
TBI5637-5MG	5 mg

### Product Details

Form: Freeze-dried powder

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Activity: 2000 U/mg protein

Protein: 50%

### Unit Definition

One unit amount of inhibitor which, after preincubation for 20 minutes at 25° C, pH 6.9 will reduce the activity of two units of human salivary alpha-amylase by 50% (one unit of alpha-amylase will liberate, from soluble starch, one micromole of maltose per minute at 25° C, pH 6.9).

### Assay Method

The reducing groups liberated from starch hydrolysis by salivary alpha-amylase, reduce 3,5-dinitrosalicylic acid resulting in formation of a colored product which can be measured spectrophotometrically at 540 nm. If the alpha-amylase inhibitor is present in the assay system it will inhibit alpha-amylase activity causing a corresponding decrease in the absorbance at 540 nm.

### Applications

A proteinaceous inhibitor of the digestive enzyme alpha-amylase (1,4-a-D-Glucan glucanohydrolase; EC 3.2.1.1) has been isolated and purified from kidney beans *Phaseolus vulgaris*. It is a glycoprotein and it specifically inhibits corresponding plant, bacterial and fungal enzymes. Maximum inhibitory activity, against alpha-amylase, occurs at 37° C and pH 5.5. The inhibitor has a molecular weight of 45,000-50,000. Similar alpha-amylase inhibitors also occur in wheat, rye and some other beans.

### Reagents

- 0.02 M Sodium phosphate buffer, pH 6.9 containing 0.006 M sodium chloride.
- 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 CO<sub>2</sub>. Stable for 2 weeks.
- 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.
- Alpha-amylase/alpha-amylase inhibitor mixture. Prepare this solution fresh. It contains human salivary alpha-amylase (1-2U), alpha-amylase inhibitor (0.5-1U), human serum albumin (1.5 mg), calcium chloride (1.5 mg) in 0.02 solution phosphate buffer, containing 0.006 M NaCl, pH 6.9. Total volume of the mixture is 1.5 ml. This mixture is preincubated for 20 minutes at 25° C prior to assay.

### Calculation

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

For research use only