Tribioscience

Trypsin Inhibitor

Catalog	Unit
TBI5636-1G	1 g
TBI5636-5G	5 g

Product Details

Form: Freeze-dried powder

Solubility: Soluble in distilled water or dilute buffer

Stability: -20° C; -4° F

Activity: 24,000 U/mg solid

Protein: 90%

Catalog No.: 114A0000

Unit Definition

The amount of inhibitor which will reduce the activity of one BAEE unit of trypsin. One BAEE unit of trypsin is the amount of enzyme causing an increase in absorbance of 0.001 per minute at 25ºC and 253 nm.

Assay Method

Trypsin hydrolyzes N-benzoyl-L-arginine ethyl ester (BAEE) at 253 nm and 25ºC. Performing this trypsin assay to obtain a known activity value for trypsin, and following with the addition of the trypsin inhibitor, will allow for the measurement of residual trypsin activity.

Applications

Trypsin inhibitors are found in a variety of sources, such as soybean, human plasma, lima bean, and bovine pancreas. Soybean trypsin inhibitor will inhibit trypsin mole for mole and will also inhibit chymotrypsinogen to a lesser extent. Trypsin inhibitor from soybean has a molecular weight of 24,000 and is composed of a single polypeptide chain, which is crosslinked by two disulfide bridges. Trypsin inhibitor is used for the treatment of hyperactive conditions of the pancreas.

Reagents

1. 0.067 M Potassium phosphate buffer, pH 7.6.
2. 0.01 M Borate buffer, pH 9.0.
3. 0.005 M HCl.
4. 0.00025 M BAEE (8.6 mg/100 ml phosphate buffer).
5. Trypsin inhibitor solution (inhibitor). Dissolve 1 mg inhibitor/ml cold borate buffer. Just prior to assay, dilute this solution to yield 2000 BAEE units inhibitor/ml borate buffer. (This dilution should now inhibit trypsin by 50%).
6. Trypsin (enzyme) solution. Dissolve 1 mg trypsin in 1 ml cold HCl. Just prior to assay, dilute solution to yield 120 BAEE units/ml HCL.

Procedure

1. Set spectrophotometer (equipped with strip chart recorder and temperature control) at 405 nm and 25ºC.
2. Perform assay of trypsin standard. Pipette 3.0 ml BAEE and 0.2 ml enzyme into cuvette. Record absorbance at 25ºC and 253 nm for 3 min. Calculate activity:
3. Use the activity results from the trypsin assay(#1) to prepare a solution of 4000 BAEE units/mo cold 0.005 M HCl.
4. Into a test tube mix 1 ml cold borate buffer and1 ml trypsin (4000 BAEE units/ml). Allow to stand in ice for 15 min. Then dilute to 1:16 to yield 250 BAEE units/ml HCl. Then assay as in (#1) for trypsin activity. Use the following calculation:
5. Into a test tube mix 1 ml inhibitor and 1 ml trypsin (4000 BAEE units/ml). Let stand for 15 min. in ice. Then dilute solution to 1:8 to yield 500 BAEE units/ml with ice cold 0.005 M HCl and assay for trypsin as in (#1). Use the following calculation:
   Continue to repeat this procedure until about 50% of the activity has been inhibited.

Calculation

Multiply the values obtained in (#4) and (#5) by BAEE units allocated to the standard, then divide this product by the value in (#2). This will adjust the values correctly. The calculation of inhibition will then be:
BAEE units of inhibition = [(corrected value for #4) - (corrected value for #5)] [inhibitor diln. per mg inhibitor]

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