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TECHNICAL INFORMATION

Catalog Number: 100612
Trypsin Inhibitor

CAS # : 9035-81-8

Synonym: Ovomuroid trypsin inhibitor

Source: Egg White

Description: Mucoprotein and antitryptic factor of egg white described by Lineweaver and Murray. ¹ Supplied as a dried powder. A glycoprotein.

Molecular Weight: 28,500 ± 3,500^{3,4,5}

Unit Description: The ability of the inhibitor to inhibit the action of bovine trypsin is measured spectrophotometrically in a system in which benzoyl-arginine-ethyl ester is utilized as the trypsin substrate.

Note: May not inhibit human trypsin.²

Activity: 1 mg inhibits approximately 2.5 - 3.0 mg of trypsin.

Assay Method:

Reagents:

- Trypsin reagent: 0.25 mM benzoyl-L-arginine ethyl ester in 0.067 M phosphate buffer, pH 7.0.
- 0.001 N Hydrochloric acid
- 0.5 M Sodium potassium phosphate buffer, pH 6.5
- Trypsin solution: prepare bovine trypsin at 0.5 mg/ml in 0.001 N HCl
- Trypsin Inhibitor solution: dissolve at 1 mg/ml in 0.001 N HCl

Procedure

Pipette the following into a series of test tubes:

Trypsin Solution	1.0 ml
0.5 M Phosphate buffer	2.0 ml
Inhibitor solution	0.1 to 0.5 ml (100 to 500 micrograms)
Reagent grade water	q.s. to 10 ml

Include 2 test tubes with no inhibitor as controls.

Incubate tubes at room temperature for 30 minutes. Dilute an aliquot with an equal portion of water and assay for residual trypsin activity. Set spectrophotometer at 25°C and 253 nm. Pipette 2.9 ml of trypsin reagent into cuvette and incubate in spectrophotometer for 3-4 minutes to achieve temperature equilibration and establish blank rate, if any. Add 0.1 ml diluted incubation mixture and record increase in A₂₅₃ for 3-4 minutes. Determine DA₂₅₃/minute from initial linear portion of curve.

Calculation:

$$\text{mg TRL inhibited/mg inhibitor} = \frac{[(\Delta A_{253}/\text{min})_{\text{control}} - (\Delta A_{253}/\text{min})_{\text{sample}}] \times 5.0^*}{(\Delta A_{253}/\text{min})_{\text{control}} \times \text{ug inhibitor in reaction mixture}}$$

* 5.0 = micrograms trypsin in reaction mixture.

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