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Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of APROTININ

PRINCIPLE:

Aprotinin will inhibit the following reaction:

BAPNA $\xrightarrow{\text{Trypsin}}$ N α -Benzoyl-DL-Arginine + p-Nitroaniline

Abbreviation used:

BAPNA = N α -Benzoyl-DL-Arginine-p-Nitroanilide

CONDITIONS: T = 25°C, pH = 7.8, $A_{405\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Triethanolamine Buffer with 20 mM Calcium Chloride, pH 7.8 at 25°C
(Prepare 100 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T1502, and Calcium Chloride, Dihydrate, Sigma Prod. No. C3881. Adjust to pH 7.8 at 25°C with 1 M NaOH.)
- B. 0.1% (w/v) N α -Benzoyl-DL-Arginine-p-Nitroanilide Solution (BAPNA)
(Prepare 25 ml in deionized water using N α -Benzoyl-DL-Arginine-p-Nitroanilide, Hydrochloride, Sigma Prod. No. B4875. Heat solution (to not greater than 65°C to facilitate solubilization). **PREPARE FRESH. THE COMPLETE SOLUBILIZATION OF SUBSTRATE IS CRITICAL.**¹⁾
- C. 1 mM Hydrochloric Acid Solution (HCl)
(Prepare 50 ml in deionized water using Hydrochloric Acid, Sigma Stock No. 920-1.)
- D. Trypsin Enzyme Solution
(Dissolve 2.5 mg of Trypsin, Type III, Sigma Prod. No. T8253, in 20 ml of cold Reagent C. **PREPARE FRESH.**)
- E. 0.9% (w/v) Sodium Chloride Solution (NaCl)
(Prepare 100 ml in deionized water using Sodium Chloride, Sigma Prod. No. S9625.)
- F. Aprotinin Inhibitor Solution
(Prepare three separate Aprotinin solutions in Reagent E, each containing 0.047 - 0.06 Trypsin Inhibitor Units per ml. Use a separate solution for each replication of assay.)²⁾

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Uninhibited</u> <u>Test</u>	<u>Inhibited</u> <u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.60	1.60	1.60
Reagent C (Hcl)	-----	-----	0.20
Reagent D (Trypsin)	0.20	0.20	-----
Reagent E (NaCl)	0.20	-----	0.20
Reagent F (Inhibitor)	-----	0.20	-----

Mix by inversion and equilibrate to 25°C. Monitor the $A_{405\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent B (BAPNA)	1.00	1.00	1.00
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Immediately mix by inversion and record the increase in $A_{405\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{405\text{nm}}/\text{minute}$ using the maximum linear rate for the Uninhibited, Inhibited, and Blank Solutions.

CALCULATIONS:

$$\text{TIU/ml} = \frac{(\Delta A_{405\text{nm}}/\text{minute Uninhibited} - \Delta A_{405\text{nm}}/\text{minute Inhibited})(\text{df})}{(9.96)(\text{ml Aprotinin/ml RM})}$$

TIU = Trypsin Inhibitor Units

df = Dilution factor

9.96 = The millimolar extinction coefficient of
p-Nitroaniline at 405 nm

RM = Reaction Mix

$$\text{TIU/mg solid} = \frac{\text{TIU/ml}}{\text{mg solid/ml}}$$

$$\% \text{ Inhibition} = \frac{\Delta A_{405\text{nm}}/\text{minute Uninhibited} - \Delta A_{405\text{nm}}/\text{minute Inhibited} \times 100}{\Delta A_{405\text{nm}}/\text{minute Uninhibited} - \Delta A_{405\text{nm}}/\text{minute Blank}}$$

UNIT DEFINITION:

One trypsin inhibitor unit (TIU) will decrease the activity of two trypsin units by 50% where one trypsin unit will hydrolyze 1.0 μmole of N α -benzoyl-DL-Arginine-p-Nitroanilide (BAPNA) per minute at pH 7.8 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 107 mM triethanolamine, 11 mM calcium chloride, 0.03% (w/v) N α -benzoyl-DL-arginine p-nitroanilide, 0.07 mM hydrochloric acid, 0.025 mg trypsin, 0.12% (w/v) sodium chloride, 0.0003% (w/v) thimerosal and 0.0094 - 0.012 trypsin inhibitor unit of aprotinin.

REFERENCES:

Fritz, H., Hartwich, G., Werle, E., (1966) *Hoppe-Seylers Zeitschrift Für Physiologische Chemie* (Berlin) **345**, 150-167

Kassell, B. (1970) *Methods in Enzymology* **XIX**, 844-852

NOTES:

1. If the solution is hazy, continue to stir over gentle heat (to not greater than 65°C) until the solution becomes clear. Do not use the solution if it turns yellow (this indicates possible chemical decomposition of the substrate due to overheating).
2. The % inhibition must be between 40 and 60 percent, for the assay to be valid. Adjust the concentration of the inhibitor solution so that the results are obtained in this range.
3. In cases where there is variability in the results, ensure that the uninhibited rate has a ΔA of 0.08 - 0.12. This may be required to reduce the variance caused by the range in specific activity of the Trypsin used.
4. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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