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ASSAY / ANALYTICAL PROCEDURE
TRYPSIN_USP

1. METHOD OF ASSAY:

That of Schwert and Takenaka in which N-benzoyl-L-arginine ethyl ester [BAEE] ishydrolysed at the ester linkage causing an increase of absorbance measured at 253nm and 25^{O}C.

$$BAEE + H_2O \xrightarrow{TRYPSIN} BA + ethanol$$

2. UNIT DEFINITION:

That amount of enzyme causing an increase in absorbance at 253nm of 0,003 per minute at 25^{O}C.

3. REAGENTS:

A **0,001M HCl**

Dilute 0,089ml concentrated HCl [MM 36,46] to 1ℓ with distilled H_2O. Store on ice.

B **0,067 M Potassium Phosphate Buffer pH7,6**

Dissolve 1,17g KH_2PO_4 [MM 136,09] and 10,08g K_2HPO_4 [MM 174,18] in distilled H_2O. Check pH7,6 and dilute to 1ℓ.

C **Substrate**

Dissolve 25,8mg BAEE . HCl [MM 342,82] / 100 ml buffer [3B] and adjust A_{253} to 0,575 versus buffer [3B]. Store at R/T.

D **Trypsin Reference/In-house Standard**

Prepare standard solution by dissolving F/D standard material in HCl [3A] at a concentration of 1mg Std/ml HCl. Immediately prior to assay dilute to yield ± 40u / ml ice-cold 0,001M HCl [3A]. i.e [ΔA_{253} / min ± 0,024] or [0,0216 ≤ ΔA_{253} / min ≤ 0,0264]

E **Sample**

Dissolve F/D Material in ice-cold HCl [3A] at a concentration of 1mg/ml **or** 5mg/ml if contaminant levels are to be determined. Immediately prior to assay dilute to yield ± 40u/ml i.e. [ΔA_{253} / min ± 0,024] or [0,0216 ≤ ΔA_{253} / min ≤ 0,0264]

4. PROCEDURE:

λ: 253nm; temp: 25^{O}C; path length: 10mm; cuvette volume: 3,2ml; sample volume: 0,2ml

Into a 10mm quartz cell, pipette the following:

Substrate [3C]	3,0 ml
Equilibrate at 25^{O}C and monitor ΔA_{253} / min.	
Enzyme [3D] or [3E]	0,2 ml
	3,2 ml

Record rate of increase in absorbance at 253nm for ± 5 minutes.

5. CALCULATION:

$$\text{Activity [u/mg material]} = \frac{\Delta A_{253} / \text{min} \times \text{dilution}}{0{,}003 \times 0{,}2 \times \text{mg enzyme/ml original solution}}$$

Where ε = 0,003 and sample volume = 0,2ml

6. COMMENTS:

6.1 Due to variations in the quality of the substrate, it is necessary to calibrate the assay system using a reference/in-house standard.

6.2 Ensure that 0,0216 ≤ ΔA_{253} / min ≤ 0,0264.

REFERENCE:

Schwert G.W. and Takenaka Y. : (1955) Biochim. Biophys ACTA 16, 570