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ASSAY / ANALYTICAL PROCEDURE
CHYMOTRYPSIN_USP

1. METHOD OF ASSAY:

As suggested by Schwert and Takenaka in which N-Acetyl-L-Tyrosine Ethyl Ester [ATEE] is hydrolyzed at the ester linkage causing a decrease of absorbance measured at 237nm and 25^{O}C.

$$\text{ATEE} + H_2O \xrightarrow{\text{Chymotrypsin}} \text{N-Acetyl-L-Tyrosine} + \text{ethanol}$$

2. UNIT DEFINITION:

That amount of enzyme causing a decrease in absorbance at 237nm of 0,0075 per minute at 25^{O}C.

3. REAGENTS:

3.1 0,067 M Potassium Phosphate Buffer pH 7,0

Dissolve 3,53 g KH_2PO_4 [MM 136,09] and 7,07 g K_2HPO_4 [MM 174,18] in ± 800 ml distilled H_2O. Check pH 7,0 and dilute to 1ℓ Store buffer at 5^{O}C.

3.2 0,001 M HCl

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

3.3 Substrate

Dissolve 70,5 mg N-Acetyl-L-Tyrosine Ethyl Ester (ATEE) [MM 251,3] in 100 ml buffer (3.1) at 70^{O}C. Cool rapidly and adjust A_{237} to 1,2 versus buffer (3.1). Store at 25^{O}C for duration of assay.

3.4 Chymotrypsin Reference/In-house Standard

Prepare standard solution by dissolving F/D standard material in HCl (3.2) at a concentration of 1 mg Std/ml HCl. Immediately prior to assay dilute to yield 10 - 20 u/ml 0,001 M HCl [$0{,}015 \leq \Delta A_{237}/\text{min} \leq 0{,}030$].

3.5 Sample

Dissolve F/D material in ice-cold HCl (3.2) at a concentration of 1mg/ml, **or** 5mg/ml if contaminant levels are to be determined. Immediately prior to assay dilute to yield 10 - 20u/ml. [$0{,}015 \leq \Delta A_{237}/\text{min} \leq 0{,}030$]

4. PROCEDURE:

Temp: 25^{O}C; λ: 237 nm; path length: 10mm; Cuvette volume: 3,2 ml; sample volume: 0,2 ml.

Into a 10 mm quartz cuvette pipette the following:

Substrate [3.3]	3,0 ml
Equilibrate at 25^{O}C and monitor ΔA_{237}/min.	
Enzyme [3.4 or 3.5] at zero time	0,2 ml
	3,2 ml

Record rate of decrease in absorbance at 237nm for ± 5 minutes.

5. CALCULATION:

$$\text{Activity [u/mg material]} = \frac{\Delta A_{237}/\text{min} \times \text{dilution}}{0{,}0075 \times 0{,}2 \times \text{mg enzyme / ml original solution}}$$

[Where ε = 0,0075 and 0,2 is enzyme volume.]

6. NOTE:

6.1 Owing to variation in substrate, the results obtained should be corrected to a reference / in-house standard [3.4]

6.2 Ensure that $0{,}015 \leq \Delta A_{237}/\text{min} \leq 0{,}030$.

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