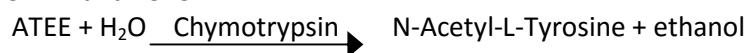


**ASSAY / ANALYTICAL PROCEDURE**  
**CHYMOTRYPSIN USP**

**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°C.



**2. UNIT DEFINITION:**

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

**3. REAGENTS:**

- 3.1 0,067 M Potassium Phosphate Buffer pH 7,0  
Dissolve 3,53 g KH<sub>2</sub>PO<sub>4</sub> [MM 136,09] and 7,07 g K<sub>2</sub>HPO<sub>4</sub> [MM 174,18] in ± 800 ml distilled H<sub>2</sub>O. Check pH 7,0 and dilute to 1ℓ Store buffer at 5°C.
- 3.2 0,001 M HCl  
Dilute 0,089 ml concentrated HCl [MM 36,46] to 1ℓ with distilled H<sub>2</sub>O. 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°C. Cool rapidly and adjust A<sub>237</sub> to 1,2 versus buffer (3.1). Store at 25°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 ≤ ΔA<sub>237</sub>/min ≤ 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 ≤ ΔA<sub>237</sub>/min ≤ 0,030]

**4. PROCEDURE:**

Temp: 25°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°C and monitor ΔA<sub>237</sub>/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 ≤ ΔA<sub>237</sub>/min ≤ 0,030.