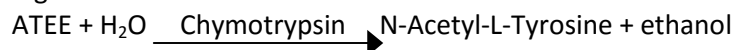


ASSAY / ANALYTICAL PROCEDURE
CHYMOTRYPSINOGEN USP

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:

0,1M Boric Acid/CaCl₂

Dissolve 6,2g Boric acid (MM 61,83) and 14,7g CaCl₂ .2H₂O (MM,147,02) in distilled water and adjust the final volume to 1,000ml using distilled water.

0,001 M HCl

Dilute 0,089 ml concentrated HCl [MM 36,46] to 1ℓ with distilled H₂O. Store on ice.

4. CHYMOTRYPSINOGEN:

Dissolve 15-20mg/ml in 0,1M Boric Acid/CaCl₂ to yield an E₂₈₀ ± 25. Record total E₂₈₀'s. Raise pH to 8,0 using 5N NaOH. (**Native activity**).

5. TRYPSIN (ACTIVATION MATERIAL):

Weigh enough Trypsin code 20010 to yield ± 5 x 10⁶ units Trypsin per 280 000 Chymotrypsinogen E₂₈₀'s.

6. CHYMOTRYPSINOGEN ACTIVATION PROCEDURE:

Add the Trypsin activation material to the Chymotrypsinogen, stir well and assay. Continue assaying every half an hour until the Chymotrypsin activity peaks (approx 2 to 4 hours). (**Potential activity**). Once peak activity is reached, kill the activation by slowly dropping the pH of the solution to pH 3,0 using 5N HCl and sample for assay.

7. ASSAY PROCEDURE:

See Chymotrypsin assay procedure.

8. CALCULATION:

See Chymotrypsin assay procedure.

Native Activity: is determined by measuring the activity of Chymotrypsin in Chymotrypsinogen prior to activation.

Potential activity: is determined from the peak Chymotrypsin activity achieved during the Chymotrypsinogen activation.