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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 and25^{O}C.

   ATEE + H_2O $\xrightarrow{\text{Chymotrypsin}}$ N-Acetyl-L-Tyrosine + 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:**
   0,1M Boric Acid/$CaCl_2$
   Dissolve 6,2g Boric acid (MM 61,83) and 14,7g $CaCl_2$.$2H_2O$ (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 H_2O. Store on ice.
4. **CHYMOTRYPSINOGEN:**
   Dissolve 15-20mg/ml in 0,1M Boric Acid/$CaCl_2$ to yield an E_{280} ± 25. Record total E_{280}'s. Raise pH to 8,0 using 5N NaOH. (**Native activity**).
5. **TRYPSIN (ACTIVATION MATERIAL):**
   Weigh enough Trypsin code 20010 to yield ± 5 x 10^6 units Trypsin per 280 000 Chymotrypsinogen E_{280}'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.