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ASSAY / ANALYTICAL PROCEDURE
TRYPSINOGEN

1. METHOD OF ASSAY:

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

$$BAEE + H_2O \xrightarrow{\text{Trypsin}} \text{N-benzoyl-L-arginine + ethanol}$$

2. UNIT DEFINITION:

That amount of enzyme causing an increase in absorbance at 253nm of 0,003 per minute at25^{O}C.

3. REAGENTS:

3.1 **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.

3.2 **0,001 M HCl**

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

4. TRYPSINOGEN(TGN):

Dissolve TGN to a concentration of 25 mg/ml in 0,1M Boric Acid/$CaCl_2$ (200 – 250mg) and record E_{280}. **Assay Native activity**.

5. TRYPSIN ACTIVATION MATERIAL:

Weigh approximately 5 mg Trypsin code 20012.

6. TRYPSINOGEN ACTIVATION PROCEDURE:

Raise pH to 8,0 using 5N NaOH. Add the Trypsin activation material to the Trypsinogen, stir well and assay. Continue assaying every half an hour until the Trypsin activity peaks (**Potential activity**), approximately 2 hours. Once peak activity is reached, kill the activationby slowly dropping the pH of the solution to pH 3,0 using 5N HCl and sample for assay.

7. ASSAY PROCEDURE:

See Trypsin assay method.

8. CALCULATION:

See Trypsin Assay Procedure

Native Activity: is determined by measuring the activity of Trypsin in Trypsinogen prior to activation.

Potential activity: is determined from the peak Trypsin activity achieved during the Trypsinogen activation.