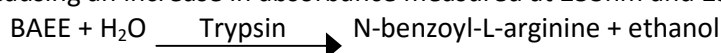


## ASSAY / ANALYTICAL PROCEDURE

### 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°C.



2. **UNIT DEFINITION:**

That amount of enzyme causing an increase in absorbance at 253nm of 0,003 per minute at 25°C.

3. **REAGENTS:**

3.1 **0,1M Boric Acid/CaCl<sub>2</sub>**

Dissolve 6,2g Boric acid [MM 61,83] and 14,7g CaCl<sub>2</sub> .2H<sub>2</sub>O [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<sub>2</sub>O. Store on ice.

4. **TRYPSINOGEN(TGN):**

Dissolve TGN to a concentration of 25 mg/ml in 0,1M Boric Acid/CaCl<sub>2</sub> (200 – 250mg) and record E<sub>280</sub>. **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 activation by 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.