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FAIZYME LABORATORIES

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ASSAY / ANALYTICAL PROCEDURE
CHYMOTRYPSIN_BP

1. METHOD OF ASSAY:

The activity of chymotrypsin is determined by comparing the rate at which it hydrolyses ethyl N-Acetyl-L-Tyrosinate with the rate at which the standard preparation hydrolyses the same substrate under the same conditions.

2. REAGENTS:

2.1 **0,001 M HCl:**

Dilute 0,089ml concentrated HCl (MM 36,46) to 1 000ml with distilled water. Store on ice.

2.2 **0,01 M $CaCl_2$:**

Dissolve 1,47g $CaCl_2.2H_2O$ (MM 147,0) or 1,11g $CaCl_2$ (MM 110,99) in 1 000ml distilled water.

2.3 **0,02 M NaOH:**

Dissolve 0,8g NaOH (MM 40,0) in 1 000ml distilled water.

2.4 **SUBSTRATE(0,2M ATEE):**

Dissolve 538,6mg N-Acetyl-L-Tyrosine Ethyl Ester [ATEE] (MM 251,3) or 577,2mg (MM 269,3) in 96% ethanol and dilute to 10ml ethanol.

2.5 **SAMPLE/REFERENCE STD:**

Dissolve 10mg F/D material in 100ml 0,001M HCl. Store on ice.

3. CALIBRATION OF pH ELECTRODE:

a) Press 'On Button' and switch on printer
b) Press up or down arrows to 'pH calibration' and 'OK'
c) Press 'continue'
d) Immerse electrode in pH4 buffer and press 'start' and 'OK'
e) Press 'next point' and 'OK'
f) Press 'end calibration' and 'OK'
g) Results will print automatically
h) Press 'Quit"

4. FILLING BURETTE (CRISON Titromatic) with 0,02M NaOH:

a) Press up or down arrows to 'Manual Activation' and 'OK'
b) Press 'Burette' and 'OK'
c) Press ^ for 'empty' and 'OK' or
d) Press ∨ to 'fill' and 'OK'
e) Press 'ESC' and 'ESC' again

5. PROCEDURE: (All operations carried out using a jacketed glass reaction vessel and water bath set at 25°C)

a) Add 30ml 0,01M $CaCl_2$ to glass reaction vessel
b) Use up or down keys and press 'Titrate' and 'OK'.
c) Use up or down keys and press 'continue' to stir
d) Once solution has equilibrated to 25°C, continue stirring (stirs ± 1minute), add 1,05ml of 0,2M ATEE.
e) Adjust pH to 8,00 with 0,02N NaOH by manually pressing 'Reagent add' and 'OK' to dispense NaOH(± 0,6ml).
f) Once the pH is 8,00 add 150µl enzyme and press 'start STAT', 'OK' and then 'start titration' and 'OK'.
g) An automatic titration is performed over 5minutes with a printout of the results.
h) A titration is carried out on the reference sample as well as the unknown sample.
i) Rinse the glass vessel after every titration, press 'continue' and 'OK' to repeat steps 5(b) to 5(f).
j) To repeat assay press "continue" and "OK".

6. CALCULATION:

$$\text{Activity [µkatal/mg]} = \frac{m' \times v \times A \times DF}{m \times v'}$$

$$\text{Activity [µkatal/mg]} = \frac{\text{mass of std} \times \text{volume of sample} \times \text{activity of std}}{\text{mass of sample} \times \text{volume of std}}$$

Where:

m = mass in mg of the test sample
m' = mass in mg of the reference sample
v = volume in ml of 0,02N NaOH used per minute by the test solution
v' = volume in ml of 0,02N NaOH used per minute by the reference solution
DF = dilution factor
A = activity of the reference in µkatal per mg

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ASSAY / ANALYTICAL PROCEDURE
DETERMINATION OF TRYPSIN IN CHYMOTRYPSIN_BP

1. REACTION:

The spot plate test is used to determine the level of trypsin in a chymotrypsin sample.

2. REAGENTS:

2.1 **Methyl Red:**

Dissolve 10 mg methyl red in 20 ml ethanol at 80% and filter if necessary. Store in an amber bottle at ambient temperature.

2.2 **Methylene Blue:**

Dissolve 20 mg methylene blue in 1 ml distilled water. Store in an amber bottle at ambient temperature.

2.3 **Methyl Red/Methylene Blue Solution:**

Mix 20ml solution 2.1 and 0,4ml solution 2.2.

2.4 **Tris (hydroxymethyl)aminomethane Buffer pH8,1:**

Dissolve 0,294 g $CaCl_2$ [MM 110,99] and 0,968 g Tris [MM 121,14] in 80 ml distilled water. Adjust pH to 8,1 and final volume to 100 ml with distilled water.

2.5 **0,0265 M $CaCl_2$ pH8,1:**

Dissolve 0,294 g $CaCl_2$ [MM 110,99] in 80 ml distilled water. Adjust pH to 8,1 and final volume to 100 ml with distilled water.

2.6 **SUBSTRATE:**

To 98,5 mg tosylarginine methyl ester hydrochloride-TAME [MM 378,88], add 5 ml tris(hydroxymethyl)aminomethane buffer pH 8,1(2.4) and dissolve. Add 2,5 ml of methyl red/methyleneblue solution(2.3) and dilute to 25 ml with distilled water. Prepare fresh with every test.

3. SAMPLES:

Dissolve sample/reference to a concentration of 10 mg/ml in distilled water.

4. PROCEDURE:

Transfer 0,05 ml $CaCl_2$ pH8,1(2.5) and 0,1 ml sample/reference onto a white spot plate. Add 0,2 ml of the substrate solution(2.6). Start the timer.

Note: If no purple colour develops within 3 minutes then not more than 1% trypsin is present in the sample.