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Carbonic Anhydrase Assay

Method

The electrometric method of Wilbur and Anderson (1948) in which the time required (in seconds) for a saturated CO2 solution to lower the pH of 0.012 M Tris· HCl buffer from 8.3 to 6.3 at 0° C is determined. The time without enzyme is recorded at T0; with enzyme, T.

Reagents

1）0.02 M Tris· HCl buffer, pH 8.0. Store in an ice bath at 0-4° C before and during use.

2）Carbon dioxide saturated water. Bubble CO2 gas through 200 ml ice cold water for 30 minutes prior to assay. During saturation process, store water at 0-4° C in an ice bath.

Enzyme

Dissolve lyophilized powder at a concentration of 0.1 mg/ml in ice cold water. Store in ice bath prior to use. IMMEDIATELY prior to use dilute suspensions or lyophilized materials to a concentration of approximately 0.01 mg/ml in ice cold water.

Procedure

1）Blank Determination: Add 6.0 ml of chilled 0.02 M Tris· HCl buffer, pH 8.0 to a 15-20 ml beaker. Maintain temperature at 0-4° C and record pH.

2）Withdraw in a 5 ml syringe, 4 ml of chilled CO2 saturated water and add to Tris buffer. Immediately start a stop watch and record the time required for the pH to drop from 8.3 to 6.3. Record this time as T0.

3）Enzyme Determination: Add 6.0 ml of chilled 0.02 M Tris· HCl buffer, pH 8.0 to a 20 ml beaker. Maintain temperature at 0-4° C and record pH. Add 0.1 ml of freshly diluted enzyme. Quickly add 4 ml of CO2 saturated water and record the time required for the pH to drop from 8.3 to 6.3. Record this time as T.

Calculation

$$\frac{\text{Units}}{\text{mg}} = \frac{2 \text{ x } (T_0 - T)}{T \text{ x mg enzyme in reaction mixture}}$$