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Lactate Dehydrogenase (LDH)

(L-Lactate: NAD+ oxidoreductase EC 1.1.1.27)

Lactate dehydrogenase (LDH) catalyzes the following reaction:

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD+} + \text{H+}$$

ASSAY

The rate of decrease in the absorbancy at 340 nm, resulting from the oxidation of NADH, is a measure of LDH activity.

REAGENTS

1. 0.1 M Sodium phosphate buffer, pH 7.0.
2. Sodium pyruvate solution, (2.5 mg/ml) in distilled water.
3. NADH solution (5 mg/ml). Dissolve 5 mg NADH, sodium salt in 1.0 ml distilled water. Always prepare fresh.
4. 1% Bovine serum albumin (BSA) solution. Dissolve 1.0 g BSA in 100 ml distilled water. Albumin should be of highest purity.
5. LDH solution (0.5-1.0 U/ml) - Dilute 0.1 ml enzyme suspension to 5 ml with cold 1% BSA solution. Use an aliquot from this stock enzyme solution and dilute to a final concentration of 0.5-1.0 U/ml with cold 1% BSA. This solution must be used as soon as it is prepared and must be made fresh for each run.

PROCEDURE

1. Set spectrophotometer (equipped with strip chart recorder and temperature control) at 340 nm and 25°C.
2. Into the cuvette, pipette the following:
   0.1 M Phosphate buffer, pH 7.0 2.7 ml
   Sodium pyruvate 0.1 ml
   NADH 0.05 ml
3. Mix and incubate at 25°C for 5 minutes.
4. Transfer the cuvette to the spectrophotometer and record the blank rate for 2-3 minutes.
5. Pipette 0.1 ml fresh enzyme solution (0.5-1.0 U/ml). Mix and monitor the reaction for 5-10 minutes at 340 nm.
6. Calculate ΔE340 nm/min

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CALCULATION

$$\text{Activity (U/mg)} = \frac{(\Delta E_{304nm/min})(\text{Total Vol.})(\text{Enz. Diln.})}{(6.22)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$