

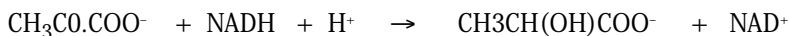
# Deacon's Challenge

## No. 33 Answer

An assay mixture for the measurement of lactate dehydrogenase constituted 2.7 mL of buffered NADH and 100  $\mu\text{L}$  of serum. The reaction was started by adding 100  $\mu\text{L}$  of sodium pyruvate. The absorbance change over 5 minutes was 0.150 when measured in a 0.5 cm light path at 340 nm. Assuming the molar absorptivity of NADH at 340 nm is  $6.30 \times 10^3 \text{ Lmol}^{-1} \text{ cm}^{-1}$ , calculate the enzyme activity.

LDH activity =  $\mu\text{mol substrate consumed/min/L plasma}$

The reaction is monitored by following the fall in absorbance at 340 nm due to the oxidation of NADH as pyruvate is consumed:



First calculate the amount of NADH oxidized over the reaction period of 5 min:

$$\Delta \text{ Absorbance} = \Delta [\text{NADH}] \text{ mol/L} \times \epsilon_{\text{NADH}} (\text{Lmol}^{-1} \text{ cm}^{-1}) \times \text{Cell path (cm)}$$

$$0.150 = \Delta [\text{NADH}] \times 6.30 \times 10^3 \times 0.5$$

$$\Delta [\text{NADH}] = \frac{0.150}{6.30 \times 10^3 \times 0.5} \text{ mol/L/5 min}$$

To convert from mol to mmol multiply by 1,000,000, and from 5 min to 1 min divide by 5:

$$\Delta [\text{NADH}] = \frac{0.150 \times 1,000,000}{6.30 \times 10^3 \times 0.5 \times 5} \mu\text{mol/L/min}$$

This is the LDH activity per litre of reaction mixture not plasma.

Since 100  $\mu\text{L}$  of plasma was diluted to a final volume of 2.9 mL (i.e. 100  $\mu\text{L}$  plasma + 2.7 mL NADH/buffer + 100  $\mu\text{L}$  of substrate) the activity (mmol/min/L plasma) is obtained by multiplying this result by 2.9 and dividing by 0.1:

$$\text{LDH activity} = \frac{0.150 \times 1,000,000 \times 2.9}{6.30 \times 10^3 \times 0.5 \times 5 \times 0.1} = 276 \text{ mmol/min/L}$$

## Question No. 34 **\*\*Christmas Special\*\***

What is the pH of a  $1.0 \times 10^{-8}$  molar solution of hydrochloric acid?

£10 book token for the first "correct" answer together with a convincing explanation received:

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