Deacon's Challenge

No 174 - Answer

Serum alkaline phosphatase activity is measured by monitoring the rate of hydrolysis of p-nitrophenyl phosphate to p-nitrophenol. p-nitrophenol has a molar absorption coefficient of 18,700 L.mol^{-1}.cm^{-1}. By convention, 1 U alkaline phosphatase is defined as the amount of enzyme that results in the formation of p-nitrophenol at a rate of 16.67 nmol per second under standard conditions.

Your laboratory analyser uses 5 µL serum diluted with 250 µL reagent in a 0.5 cm light path cuvette. Absorbance is monitored over a period of 270 seconds during which a linear increase in absorbance is expected.

Calculate the serum alkaline phosphatase activity in a sample for which the absorbance change was 0.067 absorbance units over 270 seconds.

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Use the Beer-Lambert equation for a change in absorption:

$$\Delta A = a \times b \times \Delta c$$

where ΔA = rate of absorbance change = 0.067 absorbance units/270 sec

$$= \frac{0.067}{270} \text{ absorbance units/sec}$$

a = molar absorptivity of p-nitrophenol = 18,700 L.mol^{-1}.cm^{-1}

b = light path = 0.5 cm

Δc = rate of change of concentration (mol.sec^{-1}.L^{-1})

Substitution of these values gives: $\frac{0.067}{270} = 18{,}700 \times 0.5 \times \Delta c$

Which can be re-arranged to $\Delta c = \frac{0.067}{270 \times 18{,}700 \times 0.5}$ mol/sec/L reaction mixture

Multiplication by 1,000,000,000 converts from mol to nmol

$$\Delta c = \frac{0.067 \times 1{,}000{,}000{,}000}{270 \times 18{,}700 \times 0.5} \text{ nmol/sec/L reaction mixture}$$

Multiplication by the total reaction volume and division by the sample volume allows for dilution of serum during the assay:

Total assay volume = Sample vol + Reagent vol = 5 + 250 = 255 µL

$$\text{ALP activity} = \frac{0.067 \times 1{,}000{,}000{,}000 \times 255}{270 \times 18{,}700 \times 0.5 \times 5} \text{ nmol/sec/L serum}$$

Finally, divide by 16.67 since one ALP unit is defined as 16.67 nmol/sec:

$$\text{ALP activity} = \frac{0.067 \times 1{,}000{,}000{,}000 \times 255}{270 \times 18{,}700 \times 0.5 \times 5 \times 16.67}$$

= **81 ALP units/L** (to 2 sig figs)

Question 175

A 60 mg dose of a drug is given to a male experimental subject who weighs 80 Kg. Assuming the drug is completely absorbed and distributed evenly throughout the total body water estimate the potential peak plasma level. If the drug were distributed only within the extracellular compartment what would the plasma level be?

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