Deacon's Challenge

No 149 - Answer

You are provided with the details of the alkaline phosphatase method used in your laboratory. Calculate the serum alkaline phosphatase activity in a sample for which the absorbance change was 0.073 absorbance units over 270 seconds.

Method details:
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 analyzer 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.

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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 = 0.073 absorbance units/270 sec

$$= \frac{0.073 \text{ absorbance units}}{270 \text{ sec}}$$

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})

Substituting these values gives: $\frac{0.073}{270} = 18{,}700 \times 0.5 \times \Delta c$

Which rearranges to: $\Delta c = \frac{0.073}{270 \times 18{,}700 \times 0.5}$ mol/sec/L reaction mixture

Multiplying by 1,000,000,000 to convert from mol to nmol

$$\Delta c = \frac{0.073 \times 1{,}000{,}000{,}000}{270 \times 18{,}700 \times 0.5} \text{ nmol/sec/L reaction mixture}$$

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

$$\text{ALP activity} = \frac{0.073 \times 1{,}000{,}000{,}000 \times 255}{270 \times 18{,}700 \times 0.5 \times 5} \text{ nmol/sec/L serum}$$

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

$$\text{ALP activity} = \frac{0.073 \times 1{,}000{,}000{,}000 \times 255}{270 \times 18{,}700 \times 0.5 \times 5 \times 16.67}$$

= **88 ALP units/L** (to 2 sig figs)

Question 150

A 75-year old man had a convulsion four days after a transurethral prostatectomy. He is found to have a serum sodium concentration of 105 mmol/L. His estimated weight was 64 kg. Calculate the volume of 2.7% saline required to increase his serum sodium concentration to 125 mmol/L stating any assumptions that you make (atomic weights of sodium 23, chlorine 35.5).

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