Deacon's Challenge

No 102 - 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, 1U 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.

Calculate the serum alkaline phosphatase activity in a sample for which the absorbance change was 0.076 absorbance units over 270 seconds.

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$$\Delta A = \varepsilon \times l \times \Delta c$$

Where ΔA = rate of absorbance change = 0.076 absorbance units/270 sec

$$= \frac{0.076}{270} \text{ absorbance units/sec}$$

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

l = light path = 0.5 cm

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

Substituting these values gives: $\frac{0.076}{270} = 18{,}700 \times 0.5 \times \Delta c$

Which rearranges to: $\Delta c = \frac{0.076}{270 \times 18{,}700 \times 0.5}$ mol/sec/L reaction mixture

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

$$\Delta c = \frac{0.076 \times 1{,}000{,}000{,}000}{270 \times 18{,}700 \times 0.5} \text{ nmol/sec/L reaction mixture}$$

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

$$\text{ALP activity} = \frac{0.076 \times 1{,}000{,}000{,}000 \times 255}{270 \times 18{,}700 \times 0.5 \times 5} \text{ nmol/sec/L serum}$$

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

$$\text{ALP activity} = \frac{0.076 \times 1{,}000{,}000{,}000 \times 255}{270 \times 18{,}700 \times 0.5 \times 5 \times 16.67} = \textbf{92 ALP units/L serum} \text{ (to 2 sig figs)}$$

Question 103

It is becoming increasingly common practice to replace pH with hydrogen ion concentration when reporting acid-base data. A patient is admitted to ITU with a blood hydrogen ion concentration of 80 nmol/L, pCO_2 of 5.4 kPa and actual bicarbonate of 12 mmol/L. After taking steps to improve ventilation and circulation a second set of blood gases were: pCO_2 5.1 kPa and bicarbonate 20 mmol/L.

Calculate the new hydrogen ion concentration in nmol/L.

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