

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 $18,700 \text{ L.mol}^{-1}.\text{cm}^{-1}$. 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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$$\Delta A = \epsilon \times l \times \Delta c$$

Where ΔA = rate of absorbance change = 0.076 absorbance units/270 sec
 = $\frac{0.076}{270}$ absorbance units/sec

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

l = light path = 0.5 cm

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

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

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

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

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

ACB News | Issue 558 | October 2009

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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

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:

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

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.

FRCPath, Spring 2009

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