① 2-PROPANOL + NAD → ACETONE + NADH

0.50 mL WITH 4.1 mg/L ENZYME
0.25 mL 2-PROPANOL
0.25 mL NAD

$$\text{ACTIVITY} = \frac{0.47\ \text{AU/MIN}}{6.22\ \text{AU L/mmol}} \times \frac{1000\ \mu\text{mol}}{\text{mmol}} \times \frac{1.0\ \text{mL}}{0.5\ \text{mL}} \times \frac{\text{L}}{1000\ \text{mL}}$$

$$= 0.15\ \text{IU/mL}$$

9 pts

② $$\frac{0.15\ \text{IU}}{\text{mL}} \times \frac{\text{L}}{4.1\ \text{mg}} \times \frac{1000\ \text{mL}}{\text{L}} = \frac{37\ \text{IU}}{\text{mg}}$$

6 pts

③ SEVERAL ANSWERS ARE POSSIBLE DEPENDING ON JUSTIFICATION..
THE ENZYME ASSAY SHOULD IDEALLY MEASURE V_{MAX} TO BE "REASONABLY" ACCURATE THE SUBSTRATE CONCENTRATION SHOULD BE HIGH ENOUGH TO MEASURE 90% OF V_{MAX}.

0 pts

OR $$0.90 = \frac{S}{K_m + S} \Rightarrow S \cong 19\,K_m$$

MOREOVER, THE ASSAY CALLS FOR A 4X DILUTION OF 2-PROPANOL (FROM 0.25 mL TO 1.0 mL), SO THAT THE ORIGINAL 2-PROPANOL SOLUTION SHOULD HAVE A CONCENTRATION OF $S \cong 4 \times 19\,K_m$
OR $4 \times 19 \times 4\ \text{mM} = \underline{304\ \text{mM}}$

CONCLUSION
A 300 mM 2-PROPANOL SOLUTION WILL GIVE ABOUT A 10% ERROR.

S	V	V/S
15	7.6	0.51
30	11.8	0.39
90	18.2	0.20

PLOT V ("y") VERSUS V/S ("x")

1) $K_M = \underline{35\ mM}$ (6 pts)

2) $V_{MAX} = \underline{25.3\ mmol/Ls}$ (6 pts)

3) $$k_{CAT} = \frac{(25.3\ mmol/Ls)(mol/1000\ mmol)}{(1.33\ mg/L)(g/1000\ mg)(mol/42800\ g)(2\ mol\ ACT.\ SITE/mol\ ENZ)}$$

$k_{CAT} = \underline{407{,}000\ s^{-1}}$ (6 pts)

4) $S_o - S + K_M \ln(S_o/S) = V_{MAX} t$

$$[E] = \frac{5\mu g}{0.1L} \times \frac{mg}{1000\mu g} = 0.05\ \frac{mg}{L}$$

$$V_{MAX} = \left(\frac{0.05mg}{L}\right)\left(\frac{mmol}{42800mg}\right)\left(\frac{2\ mol\ ACT\ SITE}{mol\ ENZ}\right)\left(407{,}000\ s^{-1}\right)$$

$$= 0.951\ mmol/Ls$$

$$S_0 = \frac{7 \text{ mmoles}}{0.100 L} = \frac{70 \text{ mmol}}{L}$$

$$S = 35 \frac{\text{mmol}}{L}$$

So...

$$70 - 35 + 35 \ln(70/35) = 0.951 t$$

$$t = 62 \text{ sec}$$

(6 pts)