

Calc: Units in Crude extract. $\Rightarrow$ want $\frac{\mu\text{mole}}{\text{min}}$

40 gm
 $\downarrow$ grind
 81 mL of homogenate
 $\downarrow$ centrifuge
 65 mL of supernatant.
 (80%)

$\rightarrow$ undiluted
 $\hookrightarrow \frac{1}{4}$
 $\hookrightarrow \frac{1}{10}$
 $\hookrightarrow \frac{1}{10}$

	$\Delta A/\text{min}$	$\Delta A/s$	time of assay $\downarrow$ time to zero	total Dil.
undiluted	16	.27	~ 2 sec	1
$\frac{1}{4}$	4	.067	~ 9 sec.	4x
$\frac{1}{10}$	.4	.007	~ 1.5 min	40x
$\frac{1}{10}$	.04	.0007	15 min	400

good rate. ($\Delta A/\text{min}$)

$$A = C \overset{\epsilon_{\text{app}}}{\epsilon} l$$

$$\frac{A}{\epsilon_{\text{app}}} = C \quad \Rightarrow \quad \frac{\Delta A}{\text{min}} \cdot \frac{1}{\epsilon} = \frac{\mu\text{mole}}{\text{mL} \cdot \text{min}}$$

$$\begin{aligned} \epsilon &= 6200 \text{ M}^{-1} \text{ cm}^{-1} \\ &= 6.2 \left(\frac{\mu\text{mole}}{\text{mL}} \right)^{-1} \text{ cm} \\ \epsilon_{\text{app}} &= 6.2 \left(\frac{\mu\text{mole}}{\text{mL}} \right)^{-1} \\ \text{if } l &= 1.0 \text{ cm} \end{aligned}$$

$$\frac{.04 \frac{\Delta A}{\text{min}} \times 400}{6.2 \frac{\mu\text{mole}}{\text{mL}}} = 2.58 \frac{\mu\text{mole}}{\text{mL} \cdot \text{min}} \text{ in 3 mL of assay of undiluted crude extract.}$$

$$2.58 \frac{\mu\text{mole}}{\text{mL} \cdot \text{min}} \times 3 \text{ mL}$$

$$= 7.74 \mu\text{mole}/\text{min} \text{ from } 50 \mu\text{L of undiluted crude extract}$$

$$7.74 \text{ units} / .05 \text{ mL}$$

$$= 155 \text{ units/mL of crude extract}$$

$$155 \text{ units/mL} \times 65 \text{ mL in S.F.}$$

$$= 10,062 \text{ units}$$

$$10,000 \text{ units}/.80$$

$$= 12,500 \text{ Units in crude extract.}$$

$$\text{OR } 12,500 / 4.20 = 310 \text{ Units / unit}$$

$$\text{LDH units/mL} = \Delta A/\text{min} \times 372$$

if always use 50 μL in 3.0 mL assay
 (6.2 $\times$ (30/.05))