

PROGRAM FOR ENZYME ASSAYS:

For use during LABS 3, Lab Practical, and all LDH PURIFICATION LABS

Basic Instructions:

1. Check under "TEMP" in the lower right hand corner of the screen to be sure the temperature control is "ON".
2. Click on <Kinetics/Time> in the "Routine Measure" window. Then click on <Method> at the top of the window followed by <1_cuvet> and <Exit>.
3. Place the cuvet containing the enzyme assay blank into Slot #1, wait a couple seconds, and then click <Blank>. Remember to begin every kinetics run by blanking first.
4. It is useful to be in the "Tabulation" mode (as indicated above the menu bar) in order to see the absorbance values listed as they are acquired. If you are in the "Plotting" mode (which shows a graph instead), switch to the "Tabulation" mode by clicking on <Tabulate> in the menu bar.
5. Click on <Read Samples> in the menu bar.
6. Working carefully but quickly, add 100 μl of your unknown (or a control) to a substrate tube, vortex for just an instant, pour the solution into a cuvet, place the cuvet into slot #1, close the compartment, wait 3 seconds, then click <Start>. (Or to save time, you can click with the right hand button of the mouse and then it is not necessary to point at <Start>.)
7. When the run is complete find the initial rate for the assay by clicking on <Rates> in the menu bar. The "Kinetics/Time: Rates" window will appear. This window will display a plot of your run and will list the reaction in terms of $\Delta A/\text{min}$ calculated by the computer over the full 2 minute reaction time. *Do not use this rate.* You need to inspect the data and instruct the computer to recalculate using a more appropriate interval.

After you gain some experience you may want to terminate your runs before 2 minutes are up clicking <Quit> in the "Insert Sample" box. Make sure the *top* of the hour-glass pointer is in the *middle* of the <Quit> button.
8. Decide what interval you want the computer to use in calculating the slope. Set this interval by clicking on the <0.0000> under "Initial Time" and afterward on the <120.00> under "Final Time" and then entering on the keypad the beginning time and the end of the interval over which you wish the slope to be calculated. Often the first point is anomalous. Setting the initial time to ten seconds serves to eliminate this point. Set the other end of the interval so that only the linear range is included. Once you have adjusted the interval, click <OK> and the <PrtScrn>.
9. When printing is finished, click <Exit> in the menu bar to return to the previous window, and then either <Quit> and <OK> to return to the main window, or <SaveClear> and <OK> to return to do another assay.

Specific Instructions about Lab 3:

- On page 21, do not use the <2MIN_ENZ> application. Use the above.
- For the 1:1 and *no enzyme control* assay, print out the tabulated data. Do not print out the plot. You will have to plot the tabulated data by hand or on excel for comparing exact differences in values on the graph. Slope determination is not necessary for either of these two assays. *For the 1:1, if you have enough points, you can use the linear regression tool on Excel for determining the slope. Please look at page 89 of the course reader for instructions on how to use the linear regression tool.*

I highly encourage you to begin to use Microsoft Excel for most data analysis steps throughout each lab. Your use of Excel is expected for most researchers, and health care professionals.

- You will use the plot and the spectrophotometer's regression tool to determine the initial velocity for the 1:10 and 1:15.

Specific Instructions about the Lab Practical:

- You will use the program for all enzyme assays for the 1:125 dilutions and no-enzyme control.
- You need not print out the no-enzyme control data or run the assay for the full two minutes.
- Your accuracy depends on the range you choose. Time zero is often a point that is susceptible to error because the detector has not settled. If you choose points that are beyond the initial part of the reaction, you may obtain a slope that is less than the true initial velocity. (Why?) That will affect your accuracy of the enzyme assay.