INSTRUCTIONS FOR SHIMADZU UV/VIS

for enzyme kinetics

The cart which carries the Shimadzu also has a single laminated sheet with some basic instructions as well as a blue instruction book, with more detailed instructions, which are referenced below.

In these instructions the buttons which must be pressed are printed in **Bold.** Most instructions to the machine are only executed after pressing the **enter** button.

1. Plug in power source.

2. Turn on toggle switch (on left side of machine as you face the screen). Wait until the Mode Menu appears on the screen. (a few minutes)

   For Enzyme Kinetic runs:

   - From Main Menu choose #3, Time Scan: **3 enter** (For more detail about the Time Scan Menu, see blue book pp. 4-31 to 4-37. 4-33 and 4-34 are especially useful for reading what the menu saysand the function of each menu item.)
   - 2.It will ask parameter change Y/N: **yes** (On these menus you have to press yes for each parameter you want to change.)
   - Choose #1 I = : **1 enter**

3. Type in the wave length you want to follow for your experiment; *e.g.* for tyrosinase type in **475 enter.**

4. The other parameter you probably want to change is #3, which gives you the opportunity to change the absorbance limits. You will fine tune these later (see information about data processing below), but will probably want to take your initial readings with an abs upper limit as 1.00, since readings above that are not accurate anyhow. Therefore: press **yes 3 enter 1.00 enter enter.**

   The second enter confirms that you want to keep 0.00 as lower abs.

5. Your recording time is adjusted with #4. It should be set for 60 sec. If it isn't, then press **yes 4 60 enter.**

6. When all the changes are made to the menu, you are ready to start your kinetic run. Add enzyme to your prepared cuvette, mix; put in sample cuvette holder and press **start.** The scan will appear on the screen. When it is complete a message at the top of the screen will ask Data Processing Y/N?

7. press **yes.** Choose #2, EXP (for expansion /compression) press **2 enter.** You are given the option of changed four parameters in turn, starting with the upper time limit, lower time lime, upper abs limit and lower abs limit. You will probably want to change only the upper abs limit to be close to your maximum abs reading, to maximize the precision of your initial rate measurement. Therefore press **enter enter (new upper abs value) enter enter**

8. Now the curve you want to measure is on the screen. press **copy.** and your spectrum will be printed out.

   You may now start your next run, by starting with #7 above. The abs limits automatically return to your menu settings.

GENERAL SCANNING A SPECTRUM

(blue book: 4-17 to 4-30, with 4-26/4-27 most helpful)

1. In the mode menu choose #2: **2 enter**

2. Adjust your scanning l limits by changing #1. For Dopachrome, use 200 - 800nm.

3. When ready press **start.**

4. When spectrum is complete press **copy** to get a hard copy, or you can apply data processing as for the kinetic run first before you copy.

If no one is using the machine after you, turn it off.