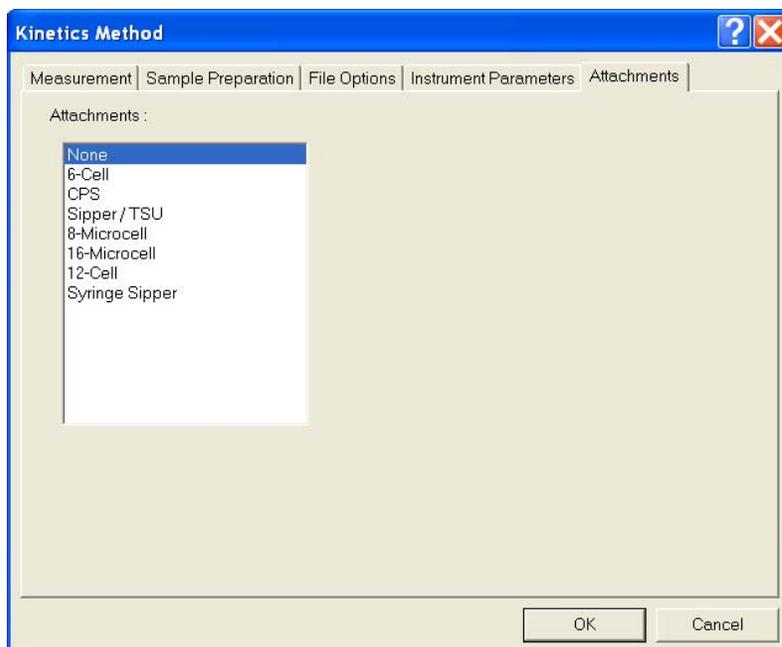


Barebones Guide to Shimadzu UV-VIS Kinetics Acquisition

1. Turn on the instrument by flipping the switch on the left-hand side. Wait about 5 minutes for the lamps to warm up.
2. Open the UV Probe software by double-clicking the icon on the desktop .
3. Click on the Connect button . The instrument will initialize and do a series of performance checks, which takes about 5-10 minutes to complete. All the boxes should be green when this is done. Click “OK” when it is complete.
4. Click on the Spectrum mode icon  or click on Windows -> Kinetics.
5. To set up your acquisition parameters, click on the Method icon  or click on Edit -> Method.
6. Click on the Attachments tab. Be sure the correct cell-holder is selected. Use “None” if you are using the standard 2-cell holder. If you are using either the 16-microcell or 12-cell holder, select the number of cells you will be acquiring data from, then click “Initialize”. *NOTE: If you are using the 16-microcell holder, you MUST select “16-Microcell” even if you are only using 8 cells!*



7. Click on the Measurement tab.

Kinetics Method

Measurement | Sample Preparation | File Options | Instrument Parameters | Attachments

Timing Mode
 Auto Manual

Units:

Total Time:

Cycle Time:

Number of Readings:

Activity Region
 Start: End:

Wavelengths (nm)
 Type: WL1: WL2:

Graph
 Y axis Upper: Y axis Lower:

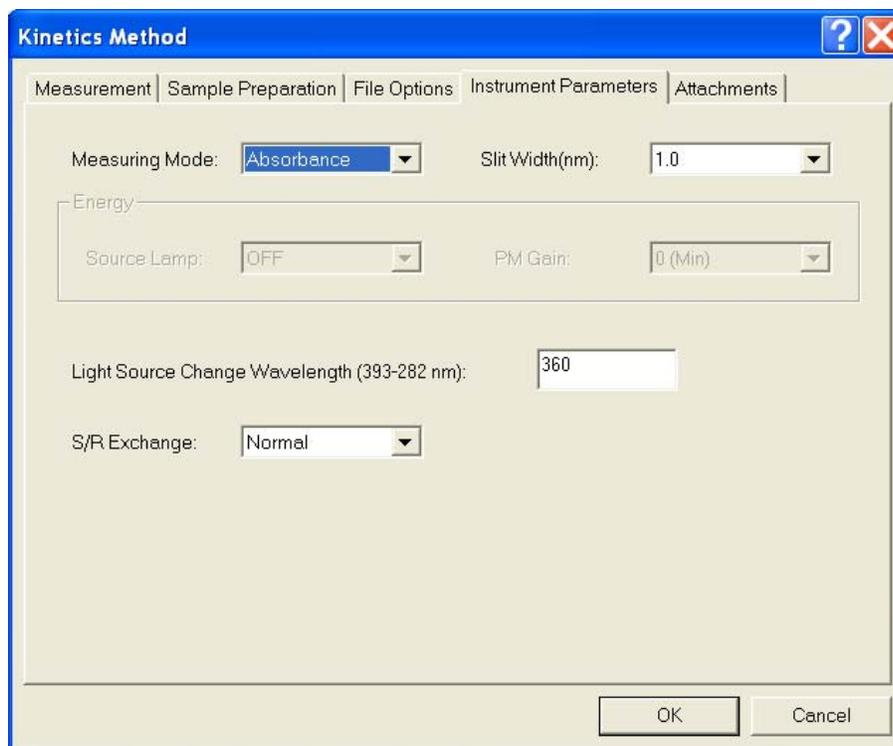
Utilities
 Enable Delay Time
 Record Events

Factors
 Factor: Correction Factor:

OK Cancel

- a. For Timing Mode, you can do one of two things:
 - i. Enter a total time in seconds and the computer will calculate the time for each cell.
 - ii. Switch to Manual Mode, where you enter how often to take a reading and how many readings you want, and the computer calculates the total time. *NOTE: If you are using the multicell holder you need to account for the time it takes the cells to move!*
 - iii. *Manual Mode Example:* Your enzyme takes about 5 minutes to fully react and you want a reading every 10 seconds. You change to Manual Mode and set the units to seconds, set the cycle time to 10 and the number of readings to 30 and the total time should calculate for you to 300.
- b. The system needs the activity region to start at 0 and end at your total time minus 1. The example above would have the activity region end at 299.
- c. On the right-hand side, change the wavelength you wish to study.

8. Click on the Instrument Parameters tab. Change the light source change wavelength if needed (ie if your wavelength is 360 nm, you may want to change the light source change wavelength to 380 nm).



9. *Optional:* Click on the Sample Preparation tab. You may enter additional information such as sample weight and dilution volume here.
10. Click on the OK button when done setting up the method.
11. Click on *cell blank*. This will remove any artifacts due to the cuvettes. It will blank for every cell portion, and may take 2-3 minutes depending on the number of cells you are using.
12. Fill your sample and reference cells with either DI water or your buffer. Click on *auto zero*.
13. Remove the cuvette filled with solvent from the frontmost cell holder(s). **When using 2-cell holder, the front cell holder is the sample holder.**
14. Place your cuvette(s) filled with sample in the front cell holder(s).
15. Click on  **Start** to start acquisition.
16. When the acquisition is complete you will be asked for a filename.

17. To autoscale your spectrum, click with the RMB on the spectrum plane and select autoscale.
18. To save your data click on File -> Save. **Data does not automatically save, you MUST save it before you exit or you lose your data!**
19. To save your data as a text file, click on File -> Save As and chose “data print table” and make sure the file extension changes to .txt.
20. When you are done acquiring all your spectra, remove and clean your cuvettes.
21. Click the  button.
22. Exit the UV Probe software.
23. Turn off the UV instrument.