

Title: Shimadzu UV-Visible Spectrophotometer SOP

Approvals:

Preparer: _____ Deb Audino _____ Date _____ 22Jul08 _____
Reviewer: _____ Bob O'Brien _____ Date _____ 22Jul08 _____

1. Purpose:

1.1. Operation of the Shimadzu UV-Visible Spectrophotometer.

2. Scope:

2.1. This machine is used to measure how much light of a given wavelength is absorbed by a liquid sample.

3. Responsibilities:

3.1. It is the responsibility of the course instructor/ lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:

4.1. Shimadzu UV-Visible Spectrophotometer manufacturer's instructions

5. Definitions:

5.1. Cuvette: A small, transparent vessel. This is what a sample is put into for testing in the UV-Visible Spectrophotometer.

6. Precautions:

6.1. N/A

7. Materials:

- 7.1. sample to be tested
- 7.2. cuvettes (visible or ultraviolet)
- 7.3. lab tissue
- 7.4. blank solution.
- 7.5. 2mL pipettes

8. Procedure:

8.1. Open panel door and make sure cuvette holders are empty, then close the panel door.

8.2. Turn spectrophotometer "ON" by flipping the yellow switch on the side of the machine.

8.3. The machine will automatically initialize and make a base line correction.

8.4. Select "8" **CONDITION SET**, then press **ENTER**.

8.5. Select "5" for **LAMP SELECT** to turn off UV light bulb, then press **ENTER**.

8.6. Press the **FILE**, Key.

8.7. Select one of the following numbers appropriate for the sample being tested.

Select "3" for **E. COLI**

Select "7" for **PICHIA**

Select "8" for **CHO**

Select "10" for **PROTEIN**

8.8. Once you have made your selection press **ENTER**.

8.9. It will then ask you for a "**Parameter Change Y/N**", Select **NO**, and press **ENTER**.

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- 8.10. Fill 2 of the same cuvettes each with about 2mL of blank solution. Hold the cuvette from the top to prevent tampering with the measurements, and wipe the sides with a lab tissue.
- 8.11. Open panel door and place the cuvettes with blank solution in the cuvette holders. **Make sure to use the appropriate orientation for the cuvettes you're using. Also make sure that the cuvettes used for the autozeroing are the same cuvette you use for the sample reading.** If using a standard cuvette, see fig 9.3, any orientation of the cuvette in the holder is acceptable, just make sure you wipe the cuvette's sides. If using a micro cuvette, see figure 9.3, the microcuvette **MUST** be oriented in the holder so the the 1cm path length goes from left to right.
- 8.12. Press the **AUTO ZERO** key, then press **ENTER**.
- 8.13. When the the Auto Zero is complete, open the panel door and remove the **front** cuvette.
- 8.14. Do not replace cuvette in rear holder.
- 8.15. Using the same cuvette style, fill an empty cuvette with about 2-ml of the sample.
- 8.16. Clean the cuvette with a lab tissue.
- 8.17. Place in front cuvette holder, using the appropriate orientation and close the panel door.
- 8.18. Press **START** to take a reading.
- 8.19. Record the results or press **COPY** for a hard copy printout.
Note: If the initial sample OD reading is greater than 1.0, the sample should be diluted until it reads below 1.0 and then multiply by the dilution factor to obtain the absorbance value.
- 8.20. Open panel door and remove test sample from front cuvette holder.
- 8.21. To test additional samples: Place cuvettes in front holder and press start for a reading.
- 8.22. Record results, or press **COPY** for a hard copy printout.
- 8.23. Press **RETURN** to bring you back to step 8.9. Note: This will erase your old data.
- 8.24. Press **FILE** to return to the original screen.
- 8.25. Remove cuvettes remaining in holders.
- 8.26. Flip power switch located on the side, to turn off the machine.

9. Attachments:

- 9.1. Figure 1: Spectrophotometer
- 9.2. Figure 2: Spectrophotometer Control Panel
- 9.3. Figure 3: Micro and Standard Cuvettes

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial release
Margaret Stiles,	2005	into 2005 SOP format
Stephen A. Derdau,	28Sep05	Amended section 8.13 regarding orientation of cuvette, and added attachments. Modified step 8.11 added the original step 8.11 to 8.11, "wipe with Kim wipe."
Deb Audino	04Apr08	Changed Kimwipe to lab tissue. College name change

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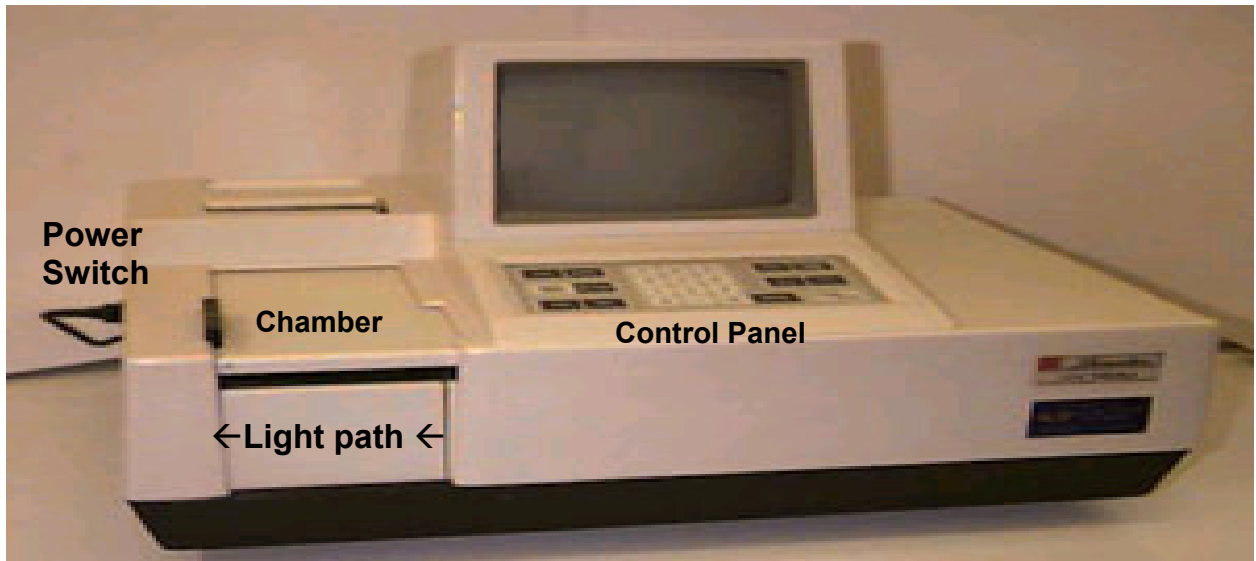


Figure 1: Spectrophotometer

Note: Light source runs from the right to the left through the sample in the cuvette chamber.



Figure 2: Spectrophotometer Control Panel

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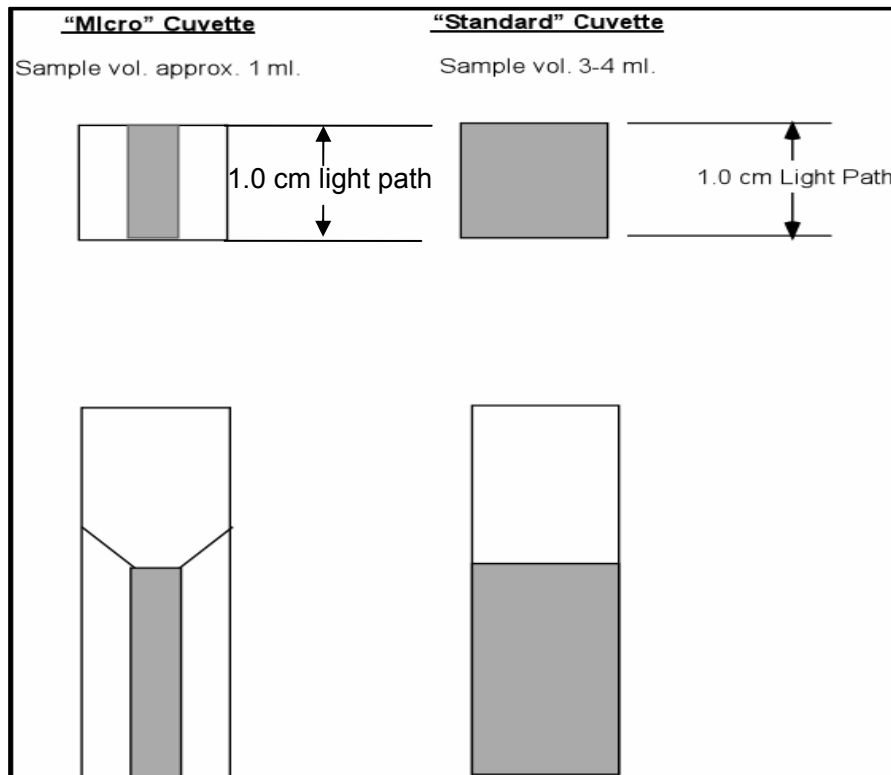


Figure 3: Micro and Standard Cuvettes

Gray Area = Sample

Image: <http://bio.classes.ucsc.edu/bio1001/A%20SPECTROSCOPY/cuvettes.pdf>