

STANDARD OPERATING PROCEDURE OF UV VISIBLE SPECTROPHOTOMETER

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	AREA: PHARMACEUTICS LAB	PAGE NO: 1-3
	SUBJECT: SOP FOR UV VISIBLE SPECTROPHOTOMETER	EFFECTIVE DATE: 25/2/2022
		REVIEW PERIOD: 25/2/2023

Purpose:

To provide a procedure for the operating of UV Visible Spectrophotometer

Scope:

Applicable to operation of UV Visible Spectrophotometer

References:

Instrument Manual

Safety Issues and Precautions:

- Wore Head cap, Mask, Hand gloves and other safety requirements during the performance of stated activity
- Please make the entry of Usage with require details in the Instrument log book
- Handle the Instrument properly with care
- After using the apparatus please clean the instrument and accessories and keep it clean
- Limit access to areas where UV sources are used.
- Cover arms and neck and limit exposure time.
- Never look directly at the beam

Procedure:

- 1) Open panel door and make sure cuvette holders are empty, and then close the panel door.
- 2) Turn spectrophotometer "ON" by flipping the yellow switch on the side of the machine.
- 3) The machine will automatically initialize and make a base line correction.
- 4) Select "**8**" **CONDITION SET**, and then press **ENTER**.
- 5) Select "**5**" for **LAMP SELECT** to turn off UV light bulb, then press **ENTER**.
- 6) Press the **FILE**, Key.
- 7) Select one of the following numbers appropriate for the sample being tested.
- 8) Select "**3**" for **E. COLI**
- 9) Select "**7**" for **PICHIA**
- 10) Select "**8**" for **CHO**
- 11) Select "**10**" for **PROTEIN**
- 12) Once you have made your selection press **ENTER**. It will then ask you for a "**Parameter Change Y/N**", Select **NO**, and press **ENTER**
- 13) 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 lab tissue.
- 14) 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 auto zeroing are the same cuvette you use for the sample reading.** If using a standard cuvette, 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 micro cuvette
- 15) Press the **AUTO ZERO** key, and then press **ENTER**.
- 16) When the Auto Zero is complete, open the panel door and remove the **front Cuvette**. **MUST** be oriented in the holder so the 1cm path length goes from left to right.

- 17) Do not replace cuvette in rear holder.
- 18) Using the same cuvette style, fill an empty cuvette with about 2-ml of the sample.
- 19) Clean the cuvette with a lab tissue.
- 20) Place in front cuvette holder, using the appropriate orientation and close the panel door.
- 21) Press **START** to take a reading.
- 22) 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.
- 23) Open panel door and remove test sample from front cuvette holder.
- 24) To test additional samples: Place cuvettes in front holder and press start for a reading.
- 25) Record results, or press **COPY** for a hard copy printout.
- 26) Press **RETURN** to bring you back to step 8.9. Note: This will erase your old data.
- 27) Press **FILE** to return to the original screen.
- 28) Remove cuvettes remaining in holders.
- 29) Flip power switch located on the side, to turn off the machine.

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