

Standard Operating Procedure for Measuring Absorbance using the Vis Spectrophotometer

SOP#: PPP-02

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1. Purpose

This procedure is for measuring absorbance of liquid bacterial culture samples using the Vis Spectrophotometer.

PLEASE NOTE: Teachers who have other types of spectrophotometers such as: 96 well plate readers, Vernier SpectroVis Plus etc, may choose to use their own equipment. Creation of an SOP for how to use alternate spectrophotometric equipment is up to the teacher.

2. Scope

This Standard Operating Procedure is intended for students using the Shoreline Biotechnology Experience Biomanufacturing Kit.

3. Definitions

Spectrophotometer: A spectrophotometer uses a light source, a lens, the sample in a cuvette, a slit, a concave mirror, and a diffraction grating to scatter the unabsorbed light onto a detection plate.

Vis: Short for wavelengths of visible light.

Cuvette: A container for use with the spectrophotometer, standardized to have a path distance of 1cm.

Transmittance: A perfectly clear object with nothing to absorb light—consider the clearest possible window—would have a transmittance of 100%. Transmittance and absorbance are inversely related.

Tare (or Blank): Using the liquid medium (typically lysogeny or LB broth) without inoculation as a baseline. By pressing abs0, the machine is told the absorbance of the liquid medium is considered to be zero, and all subsequent readings are relative to this. As a result, the absorbance of the liquid culture is assumed to be entirely due to bacterial growth.

Absorbance (A or Abs): The percent of the original light source not detected by the detection plate, presumed to have been absorbed by the molecules in the liquid sample in the cuvette. Absorbance of a material changes based on the wavelength of the light source.

Optical Density (OD): Considered a synonym for absorbance, commonly used in biology and biotechnology. The use of the word is being discouraged in favor of absorbance.

Wavelength: The distance between the crests of the light waves, in nanometers (nm). The wavelengths of visible light are 400 to 700nm. The wavelength used to measure bacterial growth is 600nm.

4. References

4.1. 2.1.5: *Spectrophotometry*. (2013, October 2). Chemistry LibreTexts.

[https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_\(Physical_and_Theoretical_Chemistry\)/Kinetics/02%3A_Reaction_Rates/2.01%3A_Experimental_Determination_of_Kinetics/2.1.05%3A_Spectrophotometry](https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Kinetics/02%3A_Reaction_Rates/2.01%3A_Experimental_Determination_of_Kinetics/2.1.05%3A_Spectrophotometry)

4.2. *Diffraction Grating*. (n.d.). Retrieved February 14, 2022, from <https://www.physics.smu.edu/~scalise/emmanual/diffraction/lab.html>

4.3. *SODIUM HYPOCHLORITE | CAMEO Chemicals | NOAA*. (n.d.). Retrieved February 14, 2022, from <https://cameochemicals.noaa.gov/chemical/4503>

5. Materials, Reagents, and Equipment

5.1 Materials:

- 5.1.1 A plastic container for holding the 10% bleach solution.
- 5.1.2 A clean cuvette supplied
- 5.1.3 Roll of paper towels on hand
- 5.1.4 Micropipette p1000 plus tips
- 5.1.5 Box of KimWipes (delicate task wipes)
- 5.1.6 Sharpie marker

5.2 Reagents:

- 5.2.1 Sterile Lysogeny Broth (LB) for blank
- 5.2.2 Bacterial Culture to be measured

5.3 Equipment:

- 5.3.1 Vis Photospectrometer

6. Responsibilities of the Quality Control Technician (QC Tech)

Lab personnel overseeing and performing the procedure are responsible for adhering to current lab safety procedures. Lab technicians must be trained to operate equipment and follow precautions listed below for handling potentially hazardous materials.

7. Hazards and Safety Precautions

7.1 Sodium Hypochlorite (bleach)

- 7.1.1 *Warning: Causes serious eye irritation, potential skin irritant*
- 7.1.2 Prevention: Wear Personal Protective Equipment (PPE), avoid contact, ingestion
- 7.1.3 Response: Flush eyes with water for up to 30 minutes after exposure, wash hands after exposure

8. Procedure

8.1 Startup and Calibration of Vis Spectrophotometer

Note: Refer to section 9 on page five for labeled images of the Vis Photospectrometer.

- 8.1.1 Turn on the Vis Spectrophotometer. It requires twenty minutes to warm up before use. The power button is on the back left-hand side of the machine.
- 8.1.2 Prepare 10% bleach solution as follows:

Mix, in a container that is about 500mL or larger, a 10% bleach solution. *Ex: If 10mL of bleach is used, add 90 mL of water.* Leave the container of 10% bleach solution in the sink.

8.1.3 Calibrate (tare) the spectrophotometer.

Note: Refer to section 9 on page five for labeled images of the Vis Photospectrometer.

- 8.1.3.1 Ensure the cuvette is clean. Wipe it free of smudges with a kimwipe.
- 8.1.3.2 After the spectrophotometer is warmed up, use the p1000 micropipette and tip to transfer **2 mL of lysogeny broth (LB)** into the cuvette. This cuvette is called the blank.
- 8.1.3.3 Open the lid of the spectrophotometer. The spectrophotometer can hold four cuvettes at a time, if necessary. The cuvettes can be moved into the optical path by manually withdrawing or pushing in the knob on the front right of the spectrophotometer. Only one cuvette is needed to calibrate the spectrophotometer.
- 8.1.3.4 Place the cuvette into the cuvette holder in the slot that is closest to the front of the machine. Make sure the knob is pushed all the way in – this should put the front-most cuvette spot in the optical path. Make sure the blank cuvette is in the optical path.
Close the lid.
- 8.1.3.5 Select the wavelength for bacterial cultures. For OD600 readings, select a wavelength of 600nm. Use the dial on the lower left of the top side of the spectrophotometer, and the dial display.
- 8.1.3.6 Select transmittance (%T) mode using the Mode button on the display panel on the upper top left of the spectrophotometer. The transmittance mode will be indicated by a light next to the label.
- 8.1.3.7 Tare by pressing the 100%T/abs button . The result should be 100% Transmittance of light through the LB. Transmittance and Absorbance are inversely related. So, the blank cuvette, which contains LB with no bacteria, should now have an Absorbance reading of zero.
- 8.1.3.8 Select the absorbance (Abs) using the Mode button on the display panel on the upper top left of the spectrophotometer. When the absorbance mode is selected you will see a light go on next to the 'Abs' label.
- 8.1.3.9 The readout should say zero. All subsequent readings will be based on the difference in absorbance between the sterile media of the blank and the media containing the growing bacteria. This equates to measuring the amount of bacteria in a solution.
Please NOTE: These spectrophotometers were not made in the USA. A reading of 0,000 is a reading of 0.000. The comma translates to a decimal point.
- 8.1.4 Remove the blank. Cap it with a small piece of plastic wrap or parafilm and set it aside in case reblanking is required. It is good practice to re-blank (other terms: recalibrate, tare) every fifteen to twenty minutes.

8.2 Collecting Absorbance Readings from Team Samples

- 8.2.1 Team QC Technicians will transfer 2 mL of the first sample to be recorded into a clean, empty cuvette.
- 8.2.2 Place the cuvette into the cuvette holder.

- 8.2.3 Repeat for up to four total samples. The cuvette holder can hold four samples at a time.
- 8.2.4 Move the cuvette of the first sample to be measured into the optical path. The cuvettes can be moved into the optical path by manually withdrawing or pushing in the knob on the front right of the spectrophotometer.
- 8.2.5 The spectrophotometer should already be set to absorbance. If not, press the mode button until the light next to *abs* is lit. Refer to
- 8.2.6 Record the reading.
- 8.2.7 Repeat as needed until all team cuvettes from each time point are measured.
- 8.2.8 Dispose of cuvette samples as follows:
 - 8.2.8.1 Pour the contents of the cuvettes into the 10% bleach solution. The contents must sit for at least fifteen minutes from the time the last cuvette or culture is added to the bleach.
 - 8.2.8.2 Reuse the cuvette for the next time point sample.
 - 8.2.8.3 Soak the cuvettes in another container of 10% bleach solution for about a minute.
 - 8.2.8.4 Drip dry for about a minute, then rinse with distilled water, wipe cuvette with Kimwipe, and set aside for reuse.
 - 8.2.8.5 After fifteen minutes, the bleach solutions can be diluted with tap water until the container is full, then poured down the drain.

8.3 Clean Station and Cuvettes

- 8.3.1 Final Cuvette cleaning
 - 8.3.1.1 Soak the cuvettes in another container of 10% bleach solution for 10 minutes.
 - 8.3.1.2 Rinse and drip dry for about a minute.
 - 8.3.1.3 Submerge cuvettes in a container of soapy water. Rinse once with tap water.
 - 8.3.1.4 Rinse with distilled water twice.
 - 8.3.1.5 Drip dry on paper towels, then put away. These cuvettes will be used again for measuring purified protein concentration.
- 8.3.2 Clean the lab bench by spraying with 70% ethanol (70% EtOH) solution, wiping evenly over all surfaces to be cleaned, then letting the solution dry on the surfaces. The time it takes the 70% EtOH to dry is approximately the time needed for the 70% EtOH to sanitize the surface. Do not spray the spectrophotometer.
- 8.3.3 Turn off the spectrophotometer.

9. Vis Photospectrometer



Figure 1: Vis Spectrophotometer

Major features starting from the upper left corner of machine:

(Upper Left) display + display indicators + control buttons;

(Lower Right) cavity with cuvette holder, knob for moving cuvettes in holder into the optical path;

(Lower Left) dial and readout for adjusting the wavelength.

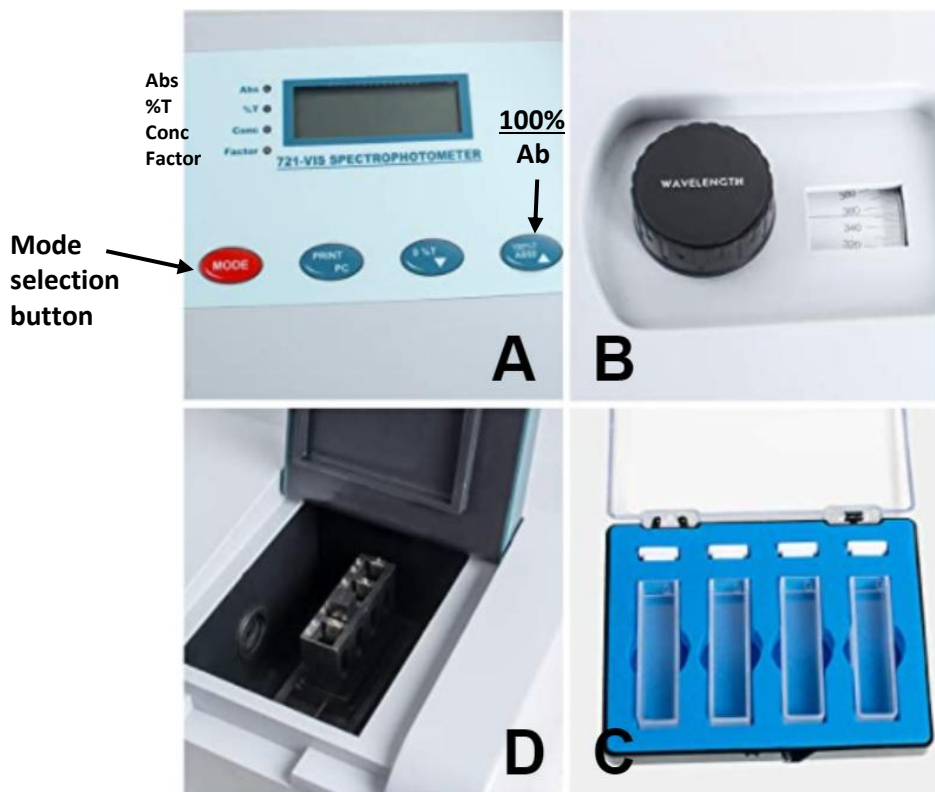


Figure 2: Features of Vis Spectrometer (clockwise from upper left):

(A) 1. Digital display top middle. 2. Indicator lights, to left of display, showing which mode is currently selected. 3. Bottom from left to right: Mode, Print PC, 0% Transmittance (T), 100% T/Abs0/Blank. (B) Dial and indicator for adjusting wavelength. This is a polychromatic device. Remember OD or absorbance readings of bacteria cultures are taken at a wavelength of 600nm. (D) Cuvette holder capacity of (4) cuvettes. Round port visible in the left middle of the interior, and the counterpart—not visible in the image—is where the light at the selected wavelength is emitted and received. (C) Clean cuvettes are stored in this container. This may differ from how cuvettes are provided and stored in the lab.