

## Directions for Use of the Vis Spectrophotometer – Teacher Document

Please refer to Figure 1 for labeled pictures of the Vis Spectrophotometer.

1. Ensure the cuvette is clean. Wipe it free of smudges with a kimwipe.
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.
3. 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 will be the blank.
4. 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.
5. 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.**
6. 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.
7. 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. 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.
9. 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.
10. 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.

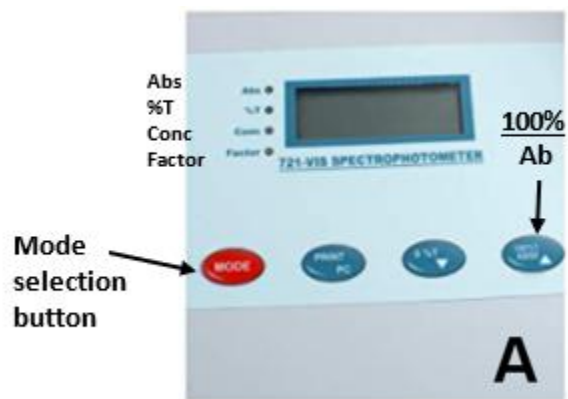


Fig 1