

Operating Instructions for the Spectrum SP-1105 Spectrophotometer

1. Set the wavelength indicator to the desired wavelength using the knob on the top of the instrument. In this case, set the wavelength to 400 nm.
2. Set the operating mode to Transmittance by pressing the **Mode** selector until the "T" is illuminated on the display.
3. You will need a pair of matched cuvettes for this experiment. Fill one of the cuvettes just over halfway with distilled water. This is your reference cuvette.
4. Open the sample compartment on the instrument (the hinged cover on the left). Wipe the reference cuvette clean with a Kimwipe, and **place it in the sample holder closest to you**. Make certain that the 'frosted' sides of the cell point towards you. Close the sample compartment. **Make sure that the knob on the lower left of the instrument is pushed all the way in at this point.**
5. Adjust the instrument to 0%T (infinite absorbance) as follows: Pull the knob on the lower left of the instrument **one notch**. Press the **0%T** button. Make certain that the instrument reads 00.0.
6. You will now set 100%T with your reference cuvette. Push the knob on the lower left back in all the way (this will move the reference cuvette into the beam.) With the knob pushed in all the way, press the **0A** button. The instrument will read **BLA** for a few seconds and then display 100.0.
7. Press the **Mode** button until the A is illuminated. You are now in Absorbance mode.
8. Open the sample compartment and remove the reference cuvette. Fill another cuvette just over halfway with the solution whose absorbance you want to measure. (In this case, fill the cuvette just over halfway with solution 10.) Wipe the cuvette clean, place it in the instrument, close the sample compartment, and record the absorbance.

Steps 1 – 8 are a general list of steps for acquiring absorbance data from the Spectrum-1105. The two steps listed below are specific to this experiment.

Using solution 10, record the absorbance every 25 nm from 400 nm up to and including 600 nm. **Remember that you must readjust 0%T and 100%T (i.e., repeat steps 1 - 7) each time you change the wavelength!**

Once you have found the maximum absorbance in this way, go back and determine λ_{max} to the nearest 5 nm by investigating wavelengths that gave the largest absorbance.

Calibration Curve and Unknown Measurement

Adjust the wavelength knob to the value of λ_{max} determined above. Don't forget to check and readjust 0%T and 100%T.

At this wavelength (λ_{max}), measure absorbance for each standard solution (solutions 1, 5, 10, and 15) as follows. With the knob on the lower left pushed all the way in:

Open the sample compartment and remove the reference solution from the sample cell holder.

Fill a cuvette just over halfway with solution 1, and place the cuvette in the sample cell closest to you.

Fill another cuvette just over halfway with solution 5, and place the cuvette in the next sample cell holder.

Place the cuvette containing solution 10 in the next sample cell.

Fill one more cuvette just over halfway with solution 15, and place the cuvette in the last sample cell holder.