Techniques - Genesys Spectrophotometer

The spectrophotometer measures the absorbance of a solution at a particular wavelength of light (the color intensity of the solution). Like an electronic balance, the spectrophotometer must be set to zero before each use. The blank is not necessarily colorless, but it is set to an absorbance of zero so that the spectrophotometer measures the difference in absorbance between the blank and experimental tubes. Absorbance is reported in Absorbance units, not nanometers.

Step by step instructions:

1. Make sure the power switch located on the rear left corner is in the “ON” position. Also be sure the instrument is set to read in absorbance (A on the right of the display).

2. Adjust the wavelength if necessary by pressing the “nm” button to move the selected wavelength the desired direction (i.e., 540 nm).

3. Prepare a blank to zero the instrument. The composition of your blank will vary depending on your experiment. Cover the top of the tube with parafilm and invert tube several times to mix contents completely. Be sure to hold the tube at the top, and wipe tube clean of fingerprints using a KimWipe.

4. Place the blank tube in the spectrophotometer, be sure the tube is installed properly (pressed up against the front of the tube holder by the spring arm), close the lid, and press the “ZERO ABS 100% T” button. The lid must be closed in order for the spectrophotometer to work.
properly. The instrument will set that absorbance as zero absorbance. Remove the blank tube.

5. Prepare the experimental tube in the same way as the blank. Mixing the tube’s components is critical. Make sure the tube is clear of any fingerprints as they will inhibit the passage of light through the tube. Place the tube in the instrument and close the lid. The absorbance will automatically be read and will be reported in the display.

The most common spectrophotometer problems occur when the spectrophotometer is not set to the proper wavelength, when tubes are not properly placed in the holder (Figure 2), when solutions are not mixed (Figure 3), and when readings are too low or too high. The spectrophotometer has a linear range where its readings are accurate. If you had a bathroom scale that read up to 200 pounds and a 350 pound person stepped on it, the scale would read 200 pounds, but that would not reflect the true weight of that individual. If your spectrophotometer readings are above about 0.5 A, you are probably not getting an accurate reading. On the other end of the scale, spectrophotometer readings below 0.02 A contain too much noise and too little signal to be considered accurate. For the best possible data, make sure that all readings are between 0.02 A and 0.5 A. If your readings are above or below these values, you may need to dilute or change reaction conditions until your sample is in the correct range.

Figure 2: A tube properly installed in the spectrophotometer.

Figure 3: A tube that has not been mixed. Notice the dark-colored solution on top and the light colored solution at the bottom of the tube. What would happen if you tried to determine the absorbance of this solution?