Vernier SpectroVis Plus Spectrophotometer Directions

**Chemicals**

Buffer, 0.2 M pH 8.0 Tris-HCl

Substrate, 0.2 M *p*-nitrophenyl phosphate in the 0.2 M pH 8.0 Tris-HCl buffer

Product, 0.000050 M *p*-nitrophenol

Enzyme solution,

Obtain 2 cuvettes.

Prepare a blank solution. Each cuvette is to hold a total of 3 mL of solution. Use an autopipet to add 100 µL of the substrate to a cuvette. Use another autopipet to add 2.9 mL of buffer to the cuvette.

# Connect SpectroVis Plus to a Computer

1. Connect the SpectroVis Plus to a USB port on the computer.
2. Double click the Logger Pro 3 icon on the desktop.

# Calibrate SpectroVis Plus

1. Choose from the “Experiment” menu Calibrate 🡪 Spectrometer 1. Wait until the system finishes warming up (90 seconds).
2. Wipe the smooth surfaces of the cuvette with Kimwipes. The software will have a dialog box saying “Place a blank cuvette in the device:”. Do so. Check that the light from the spectrometer is shining on the smooth surface of the cuvette.
3. Click “Finish Calibration”, then click OK.
4. Discard the blank solution from the cuvette and rinse with distilled water. Save the cuvette for the next steps.

# Determine the Maximum Wavelength & Set Up the Data Collection Mode

1. Rinse the cuvette twice with a small amount of the “Product” solution, then fill the cuvette ¾ full with that solution and place it in the spectrophotometer.
2. Start data collection by clicking the  button. A full spectrum graph of the solution will be displayed. Note that one area of the graph contains a peak absorbance, and there may be other lesser peaks that characterize this substance. Stop the data collection by clicking the  button.
3. Store the run by choosing from the “Experiment” menu “Store Latest Run”.
4. To set up the data collection mode, click the Configure Spectrometer button, . Click Abs vs. Time as the Collection Mode. The wavelength of maximum absorbance (λmax) will be selected. (This should be around 410 nm). Select OK. The spectrometer will display the absorbance at the selected wavelength. Record this value, which is the absorbance of the product at its λmax. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
5. Click the “Data Collection” button, , and select a “Duration” of 240 seconds, and “Sample Rate” of 0.5 samples/second (that’s 2 seconds per sample).

# Collect Absorbance-Time Data (Kinetics)

1. Remove the cuvette from the spectrophotometer and pour out the solution. Rinse the cuvette twice with a small amount of water.
2. The cuvette is to hold a total of 3 mL of solution. Add 100 µL of the substrate and 2.7 mL of buffer. 200 µL of enzyme solution is about to be added to the cuvette. As soon as enzyme is added, the reaction starts, so mixing the solution must be done quickly, which requires that a cuvette cover be **already** available. Wipe the cuvette to remove fingerprints, and handle with a Kimwipe. Add the 200 µL of enzyme solution, quickly cover it with the lid, invert a couple of times, place in the spectrometer, and immediately press the “Collect” button. The absorbance versus time will be displayed. Stop data collection if the absorbance gets to be over 1.8. (Absorbance measurements are not accurate when the absorbance is high.) If the reaction rate is too slow or fast, a different volume of enzyme solution can be used (change the amount of buffer so that the total volume of the solution is 3 mL).
3. Examine the graph of absorbance vs. time, showing a gradual increase in absorbance. Store the run by choosing Store Latest Run from the Experiment menu.
4. If required, repeat the procedure to conduct a second trial.
5. Discard the cuvette contents. Waste may go in the drain.