**Effect of pH on Peroxidase Enzyme Activity**

Materials:

* Turnip peroxidase (enzyme)
* 0.1% hydrogen peroxide (substrate)
* Guaiacol (reaction indicator)
* Distilled water
* Test tubes and rack
* Timer
* Pipettes

Procedure:

1. At the very top use a sharpie to mark one test tube “S” for substrate, another test tube “E” for enzyme, and a third test tube “B” for blank.
2. To the substrate tube, add 3.5 mL of **(pH 4, pH 7, or pH 10 buffer)**, 150 µL of 0.1 percent hydrogen peroxide (substrate), and 100 µL guaiacol (reaction indicator) for a total volume of 3.75 mL. Cover the test tube with a piece of Parafilm and gently mix.
3. To the enzyme tube, add 3 mL of **(pH 4, pH 7, or pH 10 buffer)** and 1 mL of peroxidase (enzyme) for a total volume of 4 mL. Cover the test tube with a piece of Parafilm and gently mix.
4. To the blank tube, you will add all the materials *except* the substrate. Add 6.65 mL of **(pH 4, pH 7, or pH 10 buffer)**, 100 µL of guaiacol, and 1 mL of peroxidase enzyme for a total volume of 7.75 mL.

Using the spectrophotometer

1. To measure the color change of the reaction, set the wavelength of the spectrophotometer to 470nm.
2. When the spectrophotometer is available, put the blank tube into the spectrophotometer and calibrate the absorbance to zero.
3. Combine the contents of the “S” tube and “E” tube into the “S” test tube, cover the tube with Parafilm, invert twice to mix. Start the timer.
4. Place the test tube into the spectrophotometer. Record the absorbance at 1, 2, 3, 4, 5, and 6 minutes.
5. Record your data.