Contents

Enzyme kinetics of Turnip Peroxidase

Hydrogen peroxide (H_2O_2) is a strong oxidizing agent that can damage cells and is formed as a by-product of oxygen consumption. Fortunately, aerobic cells contain peroxidases that break down peroxide into water and oxygen. This enzyme reduces hydrogen peroxide into H_2O by oxidizing an organic compound (AH_2 -> A).

This exercise uses turnip extract as a source of peroxidase. This turnip extract requires a source of electrons (a reducing agent) in order for the reaction to occur. In this case, a colorless organic compound called **guaiacol** is used. Guaiacol is oxidized in the process of converting the peroxide and becomes brown. Enzymatic activity can then be traced using a spectrophotometer to measure the amount of brown being formed.

Set-up and Calibrate LabQuest with SpectroVis Plus

1. Set-up a cuvette to serve as a Blank
    1. Add 10 drops 0.02% hydrogen peroxide
    2. Add 5 drops 0.2% guaiacol
    3. Add 20 drops of pH 7 buffer
    4. Add 10 drops extraction buffered
2. Connect a LabQuest2 to a SpectroVis plus and start the data collection program
3. Calibrate the SpectroVis
    1. Change Mode
        1. Select “Time-Based” from the dropdown menu
            - Rate: 0.5 samples/s

- Interval: 2 s/sample
- Duration: 200 s

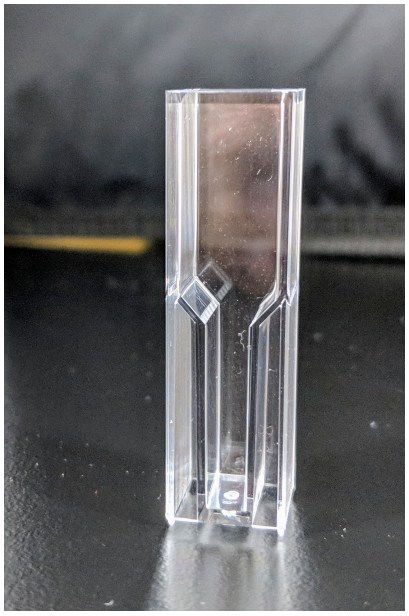
1. OK

2. Press Play button (green arrow)
    1. Perform Warm-up for 90 seconds
        1. Choose “Finish Calibration”
        2. Press “OK”
    2. Press Stop button “Red Square”

2. Select “Meter Icon” (top left most of display)
    1. Press the large red area showing the current absorbance reading
    2. Choose “Change Wavelength”
    3. Set Wavelength to 500nm

Effect of pH on Peroxidase Activity

1. Set-up cuvette for pH 3
    1. Add 10 drops 0.02% hydrogen peroxide
    2. Add 5 drops 0.2% guaiacol
    3. Add 20 drops of pH 3 buffer
    4. Add 10 drops turnip extraction last
    5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus
    6. Press play button to begin recording data (choose “discard data” if prompted)
        1. After 200s, remove SpectroVis from USB
        2. Insert a USB flash drive and wait 1 minute
        3. Press File → Export → choose USB icon and rename the file and add “.csv” to the end of the file name
        4. Press OK
2. Sequentially repeat the experiment exchanging the pH buffer with pH 5, 7, 10



Effect of Temperature on Peroxidase Activity

1. Set-up cuvette for 0ºC
    1. Add 10 drops 0.02% hydrogen peroxide (incubated at 0ºC for 10 minutes)
    2. Add 5 drops 0.2% guaiacol
    3. Add 20 drops of extraction buffer at 0ºC
    4. Add 10 drops turnip extraction (incubated at 0ºC for 10 minutes) last
    5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus
    6. Press play button to begin recording data (choose “discard data” if prompted)
        1. After 200s, remove SpectroVis from USB
        2. Insert a USB flash drive and wait 1 minute
        3. Press File → Export → choose USB icon and rename the file and add “.csv” to the end of the file name
        4. Press OK
2. Sequentially repeat the experiment exchanging the buffers stored at 20ºC, 40ºC, 60ºC

Effect of Substrate Concentration on Peroxidase Activity

1. Set-up cuvette for 1X substrate
    1. Add 10 drops 0.02% hydrogen peroxide
    2. Add 5 drops 0.2% guaiacol

3. Add 20 drops of extraction buffer
4. Add 10 drops turnip extraction last
5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus
6. Press play button to begin recording data (choose “discard data” if prompted)
    1. After 200s, remove SpectroVis from USB
    2. Insert a USB flash drive and wait 1 minute
    3. Press File → Export → choose USB icon and rename the file and add “.csv” to the end of the file name
    4. Press OK

2. Sequentially repeat the experiment with differing amounts of buffer and peroxide:
    1. Repeat the experiment with 0.2X substrate and export data to USB drive
    2. Repeat the experiment with 2X substrate and export data to USB drive
    3. Repeat the experiment with 3X substrate and export data to USB drive

	0.2X substrate	1X substrate	2X substrate	3X substrate
Drops Peroxide	2	**10**	20	30
Drops Guaiacol	5	**5**	5	5
Drops Extraction Buffer	28	**20**	10	0
Drops Extract	10	**10**	10	10

Effect of Enzyme Concentration on Peroxidase Activity

1. Set-up cuvette for 1X enzyme
    1. Add 10 drops 0.02% hydrogen peroxide
    2. Add 5 drops 0.2% guaiacol
    3. Add 20 drops of extraction buffer
    4. Add 10 drops turnip extraction last
    5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus
    6. Press play button to begin recording data (choose “discard data” if prompted)

    1. After 200s, remove SpectroVis from USB
    2. Insert a USB flash drive and wait 1 minute
    3. Press File → Export → choose USB icon and rename the file and add ".csv" to the end of the file name
    4. Press OK
2. Sequentially repeat the experiment with differing amounts of buffer and extract:
    1. Perform the experiment on 1X enzyme and export data to USB drive
    2. Repeat the experiment with 0.2X enzyme and export data to USB drive
    3. Repeat the experiment with 2X enzyme and export data to USB drive
    4. Repeat the experiment with 3X enzyme and export data to USB drive

	0.2X enzyme	1X enzyme	2X enzyme	3X enzyme
Drops Peroxide	10	**10**	10	10
Drops Guaiacol	5	**5**	5	5
Drops Extraction Buffer	28	**20**	10	0
Drops Extract	2	**10**	20	30

Tags: quantitative reasoning