

## Effect of various factors on *polyphenol oxidase* activity

### Experiment (1): Examine the chemical nature of *polyphenol oxidase*

#### Materials:

##### Chemical

Potato crude extract, biuret reagent, distilled water.

##### Equipment and Glassware

Test tubes, rack, pipette, pipette pump, water bath.

#### Protocol:

1. Label a test tube and add 1 ml of enzyme crude extract.
2. Add 2 ml of biuret reagent.

#### Results:

| Tube                                  | Observation |
|---------------------------------------|-------------|
| Enzyme crude extract + biuret reagent |             |

### Experiment (2): Test the activity of *polyphenol oxidase*

#### Materials:

##### Chemical

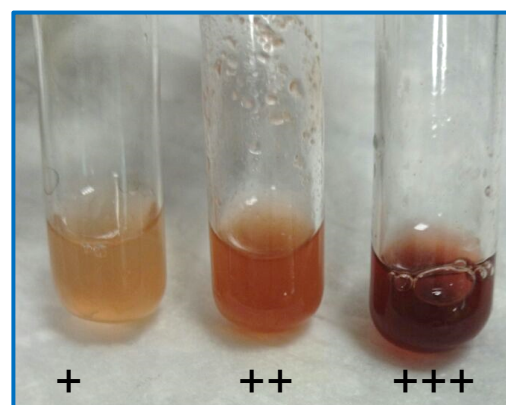
Potato crude extract, 0.01M catechol, distilled water.

##### Equipment and Glassware

Test tubes, rack, pipette, pipette pump, water bath.

#### Protocol:

1. Label 3 test tube as **A**, **B** and **C**.
2. **In tube A (control):** add 15 drops of the enzyme and 15 drops of catechol.
3. **In tube B:** add 15 drops of the enzyme and 15 drops of distilled water.
4. **In tube C:** add 15 drops of distilled water and 15 drops of catechol.
5. Shake the tubes well.
6. Place all the tubes in the water bath at 37 °C. Shake each tube every 5 minutes to aerate, thereby adding oxygen to the solution.



**Results:**

| Incubation time (Minutes) | Degree of color intensity<br>(Symbol: -, +, ++ or +++) |   |   |
|---------------------------|--|---|---|
|                           | A  | B | C |
| 0                         |  |   |   |
| 5                         |  |   |   |
| 10                        |  |   |   |
| 15                        |  |   |   |
| 20                        |  |   |   |
| 25                        |  |   |   |

**Experiment (3): Demonstrate the chemical nature of *polyphenol oxidase*****Materials:****Chemical**

Potato crude extract, 0.01M catechol, 5% TCA, phenylthiourea, distilled water.

**Equipment and Glassware**

Test tubes, rack, pipette, pipette pump, water bath.

**Protocol:**

1. Label 3 test tube as **A**, **B** and **C**.
2. **In tube A (control):** add 15 drops of the enzyme and 15 drops of catechol. Shake it.
3. **In tube B:** add 10 drops of the enzyme and 10 drops of TCA. Shake the tube thoroughly and after 5 minutes, add 10 drops of catechol.
4. **In tube C:** add 10 drops of the enzyme and few crystals of phenylthiourea. Shake the tube continually for 5 min, then add 10 drops of catechol.
5. Place all the tubes in the water bath at 37 °C for 10 minutes.
6. Compare the results obtained from B and C to the control (A).

**Results:**

| Tube        | Degree of color intensity<br>(Symbol: -, +, ++ or +++) |
|-------------|--|
| A (control) |  |
| B           |  |
| C           |  |

**Experiment (4): Investigating the substrate specificity of *polyphenol oxidase*****Materials:****Chemical**

Potato crude extract, 0.01M catechol, 0.01 M phenol, 0.01M hydroquinone, distilled water.

**Equipment and Glassware**

Test tubes, rack, pipette, pipette pump, water bath.

**Protocol:**

1. Label 3 test tube as **A**, **B** and **C**.
2. **In tube A (control)**: add 15 drops of the enzyme and 15 drops of catechol.
3. **In tube B**: add 15 drops of the enzyme and 15 drops of phenol.
4. **In tube C**: add 15 drops of the enzyme and 15 drops of hydroquinone.
5. Shake the tubes well.
6. Place all the tubes in the water bath at 37 °C for 10 minutes. Shake each tube every 5 minutes to aerate, thereby adding oxygen to the solution.

**Results:**

| Tube        | Degree of color intensity<br>(Symbol: -, +, ++ or +++) |
|-------------|--|
| A (control) |  |
| B           |  |
| C           |  |

## Experiment (5): Investigating the effect of temperature on *polyphenol oxidase* activity

### Materials:

#### Chemical

Potato crude extract, 0.01M catechol, distilled water.

#### Equipment and Glassware

Test tubes, rack, pipette, pipette pump, water bath.

### Protocol:

1. Label 3 test tube as **A**, **B** and **C**.
2. **In tube A:** add 15 drops of the enzyme and incubate at 0 °C for 10 min.
3. **In tube B:** add 15 drops of the enzyme and incubate at 37 °C for 10 min.
4. **In tube C:** add 15 drops of the enzyme and incubate at 95 °C for 10 min.
5. Add 15 drops of catechol for all tubes.
6. Shake the tubes well, then return the tubes to the proper temperature.
7. Wait for 15 minutes. Then, examine each tube without removing it from its temperature condition

### Results:

| Temperature (°C) | Degree of color intensity<br>(Symbol: -, +, ++ or +++) |
|------------------|--|
| 0                |  |
| 37               |  |
| 95               |  |