**Lab (1)**

**Some factors Affecting polyphenol Oxidase Activity**

**1- Enzymatic Activity:**

1. **Label three clean test tubes A, B and C.**
2. **Prepare each tube as follows:**

|  |  |
| --- | --- |
| **Tube** | **Addition** |
|  **A** | - 15 drops of enzyme extract.-15 drops of 0.01M catechol solution |
|  **B** | -15 drops of enzyme extract.-15 drops of distilled water.  |
|  **C** | -15 drops of 0.0M catechol solution. -15 drops of distilled water |

**c. Place all three tubes in a water bath at 37 ºC.**

|  |  |
| --- | --- |
| **Incubation time (minutes)** | **Degree of color intensity (Symbol: −, +, ++ or +++)**  |
| **TUBE A** | **TUBE B** | **TUBE C** |
| **0** |  |  |  |
| **5** |  |  |  |
| **10** |  |  |  |
| **15** |  |  |  |
| **20** |  |  |  |
| **25** |  |  |  |

**d. Shake each tube every 5 minutes to add oxygen to the solution.**

 **e. Every 5 minutes, after shaking, hold the tubes up to the light and examine.**

* **Record the color in each tube in the following table:**
* **Degree of color intensity symbol**

No color change (colorless) −

* Faint color (just detectable) +
* Definite color ++
* Dark (deep) color +++

**2-Chemical Nature Of Polyphenol Oxidase:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Tube** | **(1)** | **(2)** | **(3)** |
| **A****(Control)** |  Add 10 drops of - Enzyme extract. - 0. 01M catechol  | Shake tube and place in water bath at 37 ºC for 10 minutes.* **As a Control!**
 |
|  **B** | Add 10 drops of* Enzyme extract.
* 5% trichloroacetic acid (TCA)
 | Shake tube very well and wait 5 minutes. | - Add 10 drops of 0.01M catechol solution. -Place in water bath at 37 ºC for 10 minutes |
| **C** | * Add 15 drops of enzyme extract.
* Add a few crystals of phenylthiourea
 |

* **Examine and compare with tube A.**

|  |  |  |
| --- | --- | --- |
| **Tube**  | **Treatment** | **Degree of color intensity (Symbol: −, +, ++ or +++)** |
| **A** | **Control** |  |
| **B** | **TCA** |  |
| **C** | **Phenylthiourea** |  |

* **Record your observations in the following table**

**3-Specificity of Enzymes:**

1. ***Label three clean test tubes A, B and C.***

|  |  |  |
| --- | --- | --- |
| **Tube** | **(1)** | **(2)** |
| **A** | 15 drops of enzyme extract. | * Add 15 drops of 0.01M catechol solution
 |
| **B** | * Add 15 drops of 0.01M phenol solution
 |
| **C** | * 15 drops of 0.01M hydroquinone
 |

1. ***Shake the tubes gently and place them in a water bath at 37 ºC.***
2. ***Examine the tubes after 5 minutes and after 10 minutes.***
* ***Record the color in each tube, in the following table***

|  |  |
| --- | --- |
| **Substrate** | **Degree of color intensity (Symbol: −, +, ++ or +++)** |
| **5 minutes** | **10 minutes** |
| **Catechol**  |  |  |
| **Phenol**  |  |  |
| **Hydroquinone**  |  |  |

**4-Temperature And Enzymatic Activity:**

1. **Label three clean test tubes A, B and C.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Tube** | **(1)** | **(2) Incubate for 10 min at:** | **(3)** |
| **A** | **Add 15 drops of enzyme extract to each tube.** | **0 ºC**  | **Add 15 drops of 0.01M catechol solution to each tube.** |
| **B** | **37 ºC.**  |
| **C** | **95 ºC.**  |

1. **Shake each tube gently and quickly return it to its proper temperature condition.**
2. **Wait for 15 minutes. After this time, examine each tube, without removing it from its temperature condition**
* **Record the color in each tube in the following table.**

|  |  |
| --- | --- |
| **Temperature (ºC)** | **Degree of color intensity (Symbol: −, +, ++ or +++)** |
| **0** |  |
| **37** |  |
| **70** |  |