1. **Enzymatic Activity**

|  |  |  |  |
| --- | --- | --- | --- |
| Incubation Time (min) | Colour intensity(-, +,++ or +++) | | |
| Tube A | Tube B | Tube C |
| 0 | + | - | - |
| 5 | ++ | - | - |
| 10 | +++ | - | - |
| 15 | +++ | - | - |

1. **Chemical Nature** **of Polyphenol Oxidase**

|  |  |  |
| --- | --- | --- |
| Tube | Treatment | Colour intensity |
| A | Control | ++ |
| B | Trypsin | - |
| C | TCA | - |
| D | phenylthiourea | - |

|  |  |
| --- | --- |
| Substrate | Colour intensity |
| Catechol | +++ |
| Phenol | - |
| Hydroquinone | ++ |

**C) Substrate Specificity**

**D) Temperature and Enzymatic Activity**

|  |  |
| --- | --- |
| Temperature | Colour intensity |
| 0 ⁰C | - |
| 37 ⁰C | +++ |
| 70 ⁰C | - |

*1-Alanine Transaminase Assay*

Method:

1. Pipette 3ml of the ALT reagent
2. Pre-warm the tubes at 37 for 3 min
3. Pipette 0.2ml /200µl of serum sample
4. Mix , and allow 60 seconds for temperature equilibration
5. Read the absorbance at 340nm every minute for 3 minute /use(H2O) as blank

Results:

|  |  |  |  |
| --- | --- | --- | --- |
| ΔA/min X 1768 | Absorbance at 340nm | | Test Tube |
| ((1.656-1.621)+( 1.621-1.596))/2) X 1768 = 53.04 U/L | 1.656 | A1 | T1 |
| 1.621 | A2 |
| 1.596 | A3 |

*2-Lactate Dehydrogenase Assay*

1. Pipette 3ml of the LDH reagent
2. Pre-warm the tubes at 37 for 3 min
3. Pipette 0.4 ml/400µl of serum sample
4. Mix , and allow 60 seconds for temperature equilibration
5. Read the absorbance at 340nm every minute for 3 minute /use(H2O) as blank

Results

|  |  |  |  |
| --- | --- | --- | --- |
| ΔA/min X 4984 | Absorbance | | Test Tube |
| ((1.629-1.551)+( 1.551-1.480))/2 ) X 4984= 371.3 U/L | 1.480 | A1 | T1 |
| 1.551 | A2 |
| 1.629 | A3 |

|  |  |  |
| --- | --- | --- |
| **Tube** | **Absorbance at 405 nm** | **µmole of *P*-NP** |
| **Blank** | 0 | 0 |
| **A** | 0.099 | 5.26 |
| **B** | 0.348 | 18.51 |
| **C** | 0.933 | 49.62 |
| **D** | 0.930 | 49.49 |
| **E** | 1.027 | 54.62 |

Time

**Temperature**

|  |  |  |
| --- | --- | --- |
| **Tube** | **Absorbance at 405 nm** | **µmole of *P*-NP/min velocity** |
| **Blank** | 0 | 0 |
| **4 ⁰C** | 0.197 | 2.09 |
| **20⁰C** | 0.301 | 3.20 |
| **37 ⁰C** | 0.360 | 3.82 |
| **80 ⁰C** | 0.158 | 1.68 |
| **100 ⁰C** | 0.040 | 0.42 |

**PH**

|  |  |  |
| --- | --- | --- |
| **Tube** | **Absorbance at 405 nm** | **µmole of *P*-NP/min velocity** |
| **All Blanks** | 0 | 0 |
| **3** | 0.204 | 2.170 |
| **4** | 1.276 | 13.57 |
| **4.5** | 2.658 | 28.27 |
| **5.5** | 1.163 | 12.37 |
| **6** | 0.951 | 10.11 |
| **7** | 0.957 | 10.18 |
| **8** | 0.800 | 8.51 |