***[BCH 322]***

***The effect of temperature on the rate of an enzyme catalyzed reaction***

1. Label 14 assay tubes as the following:

|  |  |
| --- | --- |
| **Each should have its own Blank** | |
| Blank-4 Co | Test-4 Co |
| Blank-30 Co | Test-30 Co |
| Blank -37 Co | Test-37 Co |
| Blank-50 Co | Test-50 Co |
| Blank-80 Co | Test-80 Co |
| Blank-100 Co | Test-100 Co |

1. For each temperature add the following:

* **For Blank:**

|  |  |
| --- | --- |
| **Chemical** | **Volume** |
| **1.0M sodium acetate buffer (pH 5.7)** | 0.5 ml |
| **0.1M MgCl2** | 0.5 ml |
| **p-nitrophenyl phosphate** | 0.5 ml |
| **Water** | 5.5 ml |

* **For Test:**

|  |  |
| --- | --- |
| **Chemical** | **Volume** |
| **1.0M sodium acetate buffer (pH 5.7)** | 0.5 ml |
| **0.1M MgCl2** | 0.5 ml |
| **p-nitrophenyl phosphate** | 0.5 ml |
| **Water** | 5 ml |

1. Place the tubes in the labeled temperature and let the temperature equilibrate for 5 min.
2. Add 0.5 ml of enzyme extract to **TEST** and allow the reaction to proceed for 5 min.
3. Stop the reaction by the addition of 0.5ml of KOH . (Do not forget to add KOH for Blank also)
4. Repeat steps using all the water bath temperatures described in the previous table. When all of the reaction mixtures have returned to room temperature, determine the absorbance at 405 nm of each experimental tube against its own blank.

Results:

|  |  |  |
| --- | --- | --- |
| **Velocity (µmole of PNP/min)** | **Absorbance 405 nm** | **Temperature** |
|  |  | 0 |
|  |  | 30 |
|  |  | 37 |
|  |  | 50 |
|  |  | 80 |
|  |  | 100 |