***[BCH 322]***

***The effect of incubation time on the rate of an enzyme catalyzed reaction***

1. Prepare a series of **seven reaction tubes** labeled 0 through 30 minutes at 5minute intervals (Blank, 5, 10 … minutes).
2. Follow the following addition protocol for **all the tubes:**

|  |  |
| --- | --- |
| 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 all the tubes in a test rack situated in a water bath maintained at 37 ºC and let the temperature equilibrate for 5 minutes.
2. Then follow the table:

|  |  |  |
| --- | --- | --- |
| **Total incubation time (min)** | **Clock time (min)** | |
| **Start reaction (add enzyme) (0.5 ml)** | **Stop reaction (add KOH) (0.5 ml)** |
| **Note:For the blank, you must first add KOH and then the enzyme !!!** | | |
| **5** | **0** | **5** |
| **10** | **2** | **12** |
| **15** | **4** | **19** |
| **20** | **6** | **26** |
| **25** | **8** | **33** |
| **30** | **10** | **40** |

|  |  |
| --- | --- |
| **Time** | **Tube** |
| **0** | **E Tube 5** |
| **2** | **E Tube 10** |
| **4** | **E Tube 15** |
| **5** | **KOH Tube 5** |
| **6** | **E Tube 20** |
| **8** | **E Tube 25** |
| **10** | **E Tube 30** |
| **12** | **KOH Tube 10** |
| **19** | **KOH Tube 15** |
| **26** | **KOH Tube 20** |
| **33** | **KOH Tube 25** |
| **40** | **KOH Tube 30** |

1. Add 0.5ml of the enzyme to the tube marked 5 minutes, mix, start the stopwatch, and let the reaction proceed for 5 minutes before adding the KOH to terminate the reaction.
2. Run all of the other reaction tubes in exactly the same fashion with the exception that each successive tube will be incubated for 5 minutes longer than the previous one (total reaction times to equal 0, 5, 10,…30 minutes). An efficient way to do this is to start each reaction at 2-minutes intervals, keeping an eye on the stopwatch and stopping each of the reactions at the appropriate time. It is helpful to prepare a schedule of events (Table 5-1) before you begin.
3. After all the reactions have been terminated, determine the absorbance at 405 nm for each sample.

**Results:**

|  |  |  |
| --- | --- | --- |
| [P] Concentration of P-nitrophenol (µ molar) | Absorbance at405nm | Time (min) |
|  |  | **0** |
|  |  | **5** |
|  |  | **10** |
|  |  | **15** |
|  |  | **20** |
|  |  | **25** |
|  |  | **30** |