***[BCH 322]***

***The effect of substrate concentration on the rate of an enzyme catalyzed reaction***

***Method:***

* **Prepare 8 tubes labeled as follows in which each tube will contain the following concentration:**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Tube | A | B | C | D | E | F | G | H |
| [S] mM | 0 | 0.5 | 1 | 2.5 | 5 | 10 | 25 | 50 |

* **To each of these tubes add**

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

* **Place the tubes in a test tube rack situated in 37oC water bath and let stand for 5 min.**
* **Start the reaction by adding 0.5 ml enzyme and stop it by adding 0.5 ml KOH as in the following table:**

|  |  |  |
| --- | --- | --- |
| ***Tube*** | ***Start the reaction*** | ***Stop the reaction*** |
| ***A*** | ***0 min*** | ***0 min*** |
| ***B*** | ***0 min*** | ***5 min*** |
| ***C*** | ***2 min*** | ***7 min*** |
| ***D*** | ***4 min*** | ***9min*** |
| ***E*** | ***6 min*** | ***11 min*** |
| ***F*** | ***8 min*** | ***13 min*** |
| ***G*** | ***10 min*** | ***15 min*** |
| ***H*** | ***12 min*** | ***17 min*** |

***Notes:***

* (The tube containing no substrate should be used as the blank).