**BCH 447- Lab sheet**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **H2O** |  | **DNS reagent** | **0.05 mol/l phosphate buffer** | **1.2 mol/l NaOH** | **Time of hydrolysis (min)** |  | **2 mol/l HCl** | **0.05 mol/l PS buffer** | **Diluted glycogen** | **Tubes** |
| 5.5 | **Boiling water bath for 10 min**  **Cool down** | 2 | 0.5 | 1 ml | **0** | -- | 0.6 | --- | 0.4 | **1** |
| 5.5 | 2 | 0.5 | 1 ml | **4 min** | **Boiling water bath in intervals of 4 min** | 0.6 | --- | 0.4 | **2** |
| 5.5 | 2 | 0.5 | 1ml | **8 min** | 0.6 | --- | 0.4 | **3** |
| 5.5 | 2 | 0.5 | 1 ml | **12 min** | 0.6 | --- | 0.4 | **4** |
| 5.5 | 2 | 0.5 | 1 ml | **16 min** | 0.6 | --- | 0.4 | **5** |
| 5.5 | 2 | 0.5 | 1 ml | **20 min** | 0.6 | --- | 0.4 | **6** |
| 5.5 | 2 | 0.5 | 1 ml | **24 min** | 0.6 | --- | 0.4 | **7** |
| 5.5 | 2 | 0.5 | 1 ml | **28 min** | 0.6 | --- | 0.4 | **8** |
| 5.5 | 2 | 0.5 | 1 ml | **40 min** | 0.6 | --- | 0.4 | **9** |
| 5.5 | 2 | 0.5 | 1 ml | **0** | -- | 0.6 | 0.4 | --- | **Blank** |
| **Mix well ( total volume 10 ml in each tube)**  ***Read the absorbance at 540 nm against blank*** | | | | | | | | | | | |

**Acidic hydrolysis of glycogen**

**- Method:**

**Result:**

|  |  |
| --- | --- |
| **Tubes** | **Abs. at 540 nm** |
| **Blank** |  |
| **1** |  |
| **2** |  |
| **3** |  |
| **4** |  |
| **5** |  |
| **6** |  |
| **7** |  |
| **8** |  |
| **9** |  |