**A. Enzyme activity: Lab #3**

Do as following with each fraction

**1-**lable a tube as blank and fill it with distilled water, and use it for the rest of the 'enzyme activity' part of the lab.

**2-**lable another tube for fraction of interest, and add 3ml of the reagent(Lactate dehydrogenase reagent-LDH-).

**3-** add 100µl of wanted fraction(sample), mix it , and immediately turn on the stop watch.

**5-** incubate the mixture for 1 min.

**6-**reset the stop watch and read the absorbance at 340 nm each minutes for 3 min.

**7-**now do the same procedure for all other fractions.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Enzyme activity** | **∆A/min** | **∆A2** | **∆A1** | **Absorbance at 340 nm** | **minutes** | **Sample** |
|  |  |  |  |  | **1 min** | **S1**  **Crude**  **homogenate** |
|  | **2min** |
|  | **3min** |
|  |  |  |  |  | **1 min** | **S2 Supernatant homogenate** |
|  | **2min** |
|  | **3min** |
|  |  |  |  |  | **1 min** | **S3**  **40% cut supernatant** |
|  | **2min** |
|  | **3min** |
|  |  |  |  |  | **1 min** | **S4**  **65% cut supernatant** |
|  | **2min** |
|  | **3min** |
|  |  |  |  |  | **1 min** | **S5**  **65% cut resuspened pellet** |
|  | **2min** |
|  | **3min** |

**-**calculate ∆A1(A **min** 2 - A **min1**) and ∆A2(A **min** 3 - A **min2**) for each tube.

**-**Calculate the average of ∆A1and ∆A2, to get ∆A/min(∆A/min= ∆A1 + ∆A2 ).

2

**-**in order to get the activity of the enzyme in the samples, apply:

Enzyme activity=[∆A/min] X 4984= U/L

**B. Determination of protein concentration by Bradford method in different fractions:**

**1-**Set 6 test tubes, and label them as follow:

Blank, S1:Crude homogenate, S2: Supernatant of homogenate, S3: 40% cut supernatant, S4:65% cut supernatant, S5: 65% cut resuspened pellet.

**2 -**now follow the table:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Absorbance at 595 nm** | **Incubate for 5 min at room temperature** | **Volume of Bradford reagent(ml)** | **Volume of water**  **(ml)** | **Volume of the fraction (ml)** |  |
|  | 5 ml | 0.1ml | - | **blank** |
|  | 5 ml | - | 0.1ml | **S1** |
|  | 5 ml | - | 0.1ml | **S2** |
|  | 5 ml | - | 0.1ml | **S3** |
|  | 5 ml | - | 0.1ml | **S4** |
|  | 5 ml | - | 0.1ml | **S5** |

-take the concentration of each tube from previous standard curve[Lab#2].

-calculations of specific activity:

specific activity=enzyme activity/concentration of protein[U/mg].

|  |  |  |
| --- | --- | --- |
| **Cell fractions** | **Concentration of protein (µg/ml)** | **Specific activity**  **[U/mg]** |
| **S1** |  |  |
| **S2** |  |  |
| **S3** |  |  |
| **S4** |  |  |
| **S5** |  |  |