**Experiment No.1**

**Some factors affecting polyphenol oxidase activity**

**(Introduction to Enzymology)**

**Objective:**

1-To demonstrate the activity of the enzyme polyphenol oxidase in a crude extract prepared from potato.

2-To demonstrate the chemical nature of the enzyme.

3-To investigate the substrate specificity of the enzyme.

4-To investigate the effects of various temperatures on the activity of the enzyme.

**Materials:**

-0.01M catechol

-0.1M NaF

-0.01M hydroquinone

-0.01M phenol

-5% trichloroacetic acid

-5% trypsin

-Phenylthiourea

-Potatoes

**Glassware and Instrumentation:**

-Test tubes

-Pasture pipette

-Homogenizer

-Cheesecloth

-Water baths at 37 and 70 ºC

-Container of crushed ice

**Preparation Of solutions:**

|  |  |
| --- | --- |
| **Solutions**  | **Preparation** |
| 0.01M catechol | Dissolve 1.1g of catechol in 1 liter of distilled water. Adjust the pH to 6.0 with dilute NaOH to prevent auto oxidation. Keep this solution in a brown bottle in a refrigerator. (If the solution turns brown it is unfit for use.) |
| 0.1M NaF | Dissolve 4.2g of sodium fluoride in 1 liter of distilled water. |
| 0.01M hydroquinone | Dissolve 0.11g of hydroquinone in 100ml of distilled water. |
| 0.01M phenol | Dissolve 94mg of phenol in 100ml of distilled water. |
| 5% trichloroacetic acid | Dissolve 5g of trichloroacetic acid in 100ml of distilled water. |
| 5% trypsin | Dissolve 5g of trypsin in 100ml of distilled water. |
| Phenylthiourea | A few grams of crystalline phenylthiourea should be available for the experiment. |

**Method**

**Test tube enzymatic activity:**

1. Label three clean test tubes A, B and C.
2. b) Prepare each tube as follows:

Tube A: 15 drops of enzyme extract. 15 drops of 0.01M catechol solution. Tube B: 15 drops of enzyme extract. 15 drops of distilled water.

 Tube C: 15 drops of 0.0M catechol solution. 15 drops of distilled water.

 c) Place all three tubes in a water bath at 37 ºC.

d) Shake each tubes every 5 minutes to aerate, thereby adding oxygen to the solution.

e) Every 5 minutes, after shaking, hold the tubes up to the light and examine. Record the color in each tube, Continue for 25 minutes.

**Chemical nature of polyphenol oxidase:**

1. Label four clean test tubes A, B, C and D.
2. b) Prepare, and treat, each tube as follows:

Tube A: Add 15 drops of enzyme extract. Add 15 drops of 0.01M catechol solution. Shake tube and place in water bath at 37 ºC for 10 minutes.

Set tube aside as control with which to compare results of tubes B, C and D. Tube B: Add 10 drops of enzyme extract. Add 10 drops of 5% trypsin solution.

Shake tube thoroughly. Place tube in a water bath at 37 ºC for 10 minutes. Add 10 drops of 0.01M catechol solution.

Replace in the same water bath for 10 minutes.

Examine and compare with tube A. Record your observations

Tube C: Add 10 drops of enzyme extract. Add 10 drops of 5% trichloroacetic acid. Shake tube thoroughly and wait 5 minutes. Add 10 drops of 0.01M catechol solution. Place tube in water bath at 37 ºC for 10 minutes. Examine and compare with tube A. Record your observations

Tube D: Add 15 drops of enzyme extract. Add a few crystals of phenylthiourea. Shake tube thoroughly and continue shaking it frequently during a period of 5 minutes. Then add 15 drops of 0.01M catechol solution. Place tube in water bath at 37 ºC for 10 minutes. Examine and compare with tube A. Record your observations.

**Substrate specificity:**

a) Label three clean test tubes A, B and C.

b) Add 15 drops of enzyme extract to each tube.

 c) Prepare each tube as follows:

Tube A: Add 15 drops of 0.01M catechol.

Tube B: Add 15 drops of 0.01M phenol solution.

 Tube C: Add 15 drops of 0.01M hydroquinone solution.

d) Shake the tubes gently and place them in a water bath at 37 ºC.

e) Examine the tubes after 5 minutes and after 10 minutes. Record the color in each tube.

**Temperature and enzymatic activity:**

a) Label three clean test tubes A, B and C.

b) Add 15 drops of enzyme extract to each tube.

c) Place each tube containing the enzyme extract, in a water bath for 10 minutes at the following temperatures:

Tube A: 0 ºC (in a container of crushed ice). Tube B: 37 ºC. Tube C: 70 ºC.

d) Add 15 drops of 0.01M catechol solution to each tube.

e) Shake each tube gently and quickly return it to its proper temperature condition.

Wait for 15 minutes. After this time, examine each tube, without removing it from its temperature condition, and record the color in each tube.

1. **Enzymatic Activity**

|  |  |
| --- | --- |
| Incubation Time (min) | Colour intensity(-, +,++ or +++) |
| Tube A | Tube B | Tube C |
| 0 | + | - | - |
| 5 | ++ | - | - |
| 10 | +++ | - | - |
| 15 | +++ | - | - |
| 20 | +++ | - | - |
| 25 | +++ | - | - |

1. **Chemical Nature** **of Polyphenol Oxidase**

|  |  |  |
| --- | --- | --- |
| Tube | Treatment | Colour intensity |
| A | Control | ++ |
| B | Trypsin | - |
| C | TCA | - |
| D | phenylthiourea | - |

1. **Substrate Specificity**

|  |  |
| --- | --- |
| Substrate | Colour intensity |
| Catechol | +++ |
| Phenol | - |
| Hydroquinone | + |

1. **Temperature and Enzymatic Activity**

|  |  |
| --- | --- |
| Temperature | Colour intensity |
| 0 ⁰C | - |
| 37 ⁰C | +++ |
| 70 ⁰C | - |

**Experiment No.2**

**Effect of Time Incubation on Enzyme Activity**

**Objective:**

1-To study the effect of time on the rate of enzyme catalyzed reaction.

2- To calculate the initial velocity of the reaction, vi.

**Material:**

 - 0.05M Sodium Acetate buffer, pH 4.7

 - 0.18 M Sucrose

 -0.005M D-Glucose, 0.005M Fructose

 -DNS (dinitrosalicylicacid) Reagent

 - Beta Fructofuranosidase(Invertase ) enzyme extract from yeast

 -0.1M Sodium Bicarbonate

 -Yeast

**Glassware and Instrumentation:**

\_ Test tubes

- Pipettes

-Measuring cylinder (20ml)

- Water baths (40°, 100°)

-Spectrophotometer

-Aluminium foil.

**Preparation Of solutions:**

|  |  |
| --- | --- |
| **Solution** | **Preparation** |
|  0.05M Sodium Acetate buffer , pH 4.7  | Weight carefully 1.903g of sodium acetate (CH3COONa) , dissolve in a small volume of distilled water , then add to it 1.5ml of 99.7% Glacial Acetic acid , transfer all to a 1L volumetric flask then make up the volume to 1L by adding distilled water. Then check pH , and adjust if necessary . |
| 0.18 M Sucrose  | Weight carefully 61.614 grams of sucrose dissolve in a small volume of water , transfer to a 1L volumetric flask , then make up to 1L by adding water , mix well . |
| 0.005Mglucose,0.005M fructose. | Weight carefully 0.90gram of glucose and 0.90gram of fructose , dissolve in a small volume of distilled water , transfer to a 1Lvolumetric flask then make up to 1L by adding distilled water , mix well . |
| DNS (dinitrosalicylicacid ) Reagent . | -Solution “A” Weight carefully 6.25 grams of DNS , and 20 grams of NaOH , then dissolve in a small volume of water (to dissolve heat on a hot plate ), transfer to a 500 ml volumetric flask and add water up to the 500ml mark , mix well.-Solution “B” Weight carefully 300 grams of Putasium Sodium Tartarrate , dissolve in a small volume of water , transfer to a 500 ml volumetric flask then make up to 500ml by adding water , mix well .To Prepare the DNS Reagent : Take 222.2 ml of solution “A” add to it 277.7 ml of solution “B” mix well , (heat on a hot plate when needed ).  |
| 0.1M Sodium Bicarbonate . | Weight carefully 0.840 gram of sodium bicarbonate dissolve in a small volume of water , transfer to a 100ml volumetric flask, make up the volume to 100ml by adding water , mix well . |
| Beta Fructofuranosidase(Invertase ) enzyme extract from yeast. | Weight carefully 10grams of dry yeast dissolve in 30 ml of a 0.1M sodium bicarbonate solution , mix by magnetic stirrer for 2 hours at room temperature , then centrifuge at 3000 rpm for 10 min , collect the supernatant carefully and label as enzyme extract . Just before start of experiment prepare a diluted enzyme extract solution 1:200 by taking 1.0ml of concentrated enzyme extract and adding water up to 200ml in a volumetric flask mix gently . |

**A- Preparation of Calibration Curve for Reducing Sugars**

**Method:**

1. Prepare 7 test tubes in the following manner , table ( 1 ):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tube | 0.005M Standard reducing sugars (ml) | Water .(ml) | Acetate buffer (ml) | DNS reagent (ml) |
| Blank | - | 2.0 | 1.0 | 2.0 |
| A | 0.2 | 1.8 | 1.0 | 2.0 |
| B | 0.4 | 1.6 | 1.0 | 2.0 |
| C | 0.8 | 1.2 | 1.0 | 2.0 |
| D | 1.0 | 1.0 | 1.0 | 2.0 |
| E | 1.5 | 0.5 | 1.0 | 2.0 |
| F | 2.0 | - | 1.0 | 2.0 |

2-Mix each tube properly then, cover each tube with aluminium foil and place in a boiling water bath for 5 min

3-Remove the tubes from the water bath , cool under tap water , then add 20ml of distilled water to each tube and mix properly .

4-Measure the absorbance of each tube against the blank at 540nm, then record the absorbance in the following table (2).

5-Construct the calibration curve by plotting the absorbance at 540nm against the Concentration of reducing sugars in µMolar

Table (2)

|  |  |  |  |
| --- | --- | --- | --- |
| Tube | Absorbance 540nm | Concentration of reducing sugars M  | Concentration of reducing sugars µM |
| A | 0.06 | 5x10-4 | 500 |
| B | 0.112 | 1.0 x 10-3 | 1000 |
| C | 0.299 | 2.0 x 10-3 | 2000 |
| D | 0.307 | 2.5 x 10-3 | 2500 |
| E | 0.471 | 3.75 x 10-3 | 3750 |
| F | 0.622 | 5.0 x 10-3 | 5000 |

**Standard Graph for reducing sugars**

 **B-Effect of Time Incubation on the Rate of an Enzymatic Reaction**

**Method:**

1- Prepare 8 test tubes in the following manner table (3):

|  |  |  |
| --- | --- | --- |
| Tube | Acetate buffer (ml) | 0.18M Sucrose (ml) |
| Blank | 1.0 | 2.0 |
| A | 1.0 | 2.0 |
| B | 1.0 | 2.0 |
| C | 1.0 | 2.0 |
| D | 1.0 | 2.0 |
| E | 1.0 | 2.0 |
| F | 1.0 | 2.0 |

2- Mix each tube then add 0.05ml of diluted enzyme except for the blank add 0.05ml of distilled water instead , mix and start the stop clock immediately , incubate each tube according to the following table (4) , then stop the reaction by adding 2.0ml of the DNS reagent to each tube .

Note: Mix each tube frequently during the incubation time

Table (4)

|  |  |  |  |
| --- | --- | --- | --- |
| Tube  | Start Time (min) | Stop by adding 2.0ml DNS (min) | Incubation time |
| Blank | 0 | 0 |  |
| A | 1.0 | 6 | 5 |
| B | 2.0 | 12 | 10 |
| C | 3.0 | 23 | 20 |
| D | 4.0 | 34 | 30 |
| E | 5.0 | 45 | 40 |
| F | 6.0 | 56 | 50 |
| G | 7.0 | 67 | 60 |

3- Mix properly, cover each tube by aluminium foil and place in a boiling water bath for 5min to allow the colour to develop.

4- Then remove from water bath cool under tap water, add 20ml of distilled water to each tube, mix properly then measure the absorbance at 540nm.

5- Record the absorbance of each test tube in the following table (5),

6- Convert the Absorbance reading obtained to micromoles of sucrose hydrolyzed making use of the standard reducing sugars calibration curve.

7- Obtain the relationship between the micromoles of sucrose hydrolyzed and Time in min. Then determine the initial velocity of the reaction.

Table (5)

|  |  |  |
| --- | --- | --- |
| Tube | Absorbance 540nm | µmoles of sucrose hydrolyzed |
| A | 0.199 | 800 |
| B | 0.247 | 2000 |
| C | 0.427 | 3550 |
| D | 0.502 | 4150 |
| E | 0.476 | 3900 |
| F | 0.552 | 4550 |
| G | 0.587 | 4850 |

**Graph for Effect of Incubation Time on Enzyme Activity**



**Experiment No.3**

**Effect of Enzyme Concentration on the Rate of an Enzymatic Reaction**

**Objective:**

1. To establish the relationship between enzyme concentration and the rate of an enzyme catalyzed reaction.

**Preparation Of solutions:**

|  |  |
| --- | --- |
| **Solution** | **Preparation** |
|  0.05M Sodium Acetate buffer , pH 4.7  | Weight carefully 1.903g of sodium acetate (CH3COONa) , dissolve in a small volume of distilled water , then add to it 1.5ml of 99.7% Glacial Acetic acid , transfer all to a 1L volumetric flask then make up the volume to 1L by adding distilled water. Then check pH , and adjust if necessary . |
| 0.18 M Sucrose  | Weight carefully 61.614 grams of sucrose dissolve in a small volume of water, transfer to a 1L volumetric flask, then make up to 1L by adding water, mix well. |
| 0.005Mglucose, 0.005M fructose. | Weight carefully 0.90gram of glucose and 0.90gram of fructose, dissolve in a small volume of distilled water, transfer to a 1Lvolumetric flask then make up to 1L by adding distilled water, mix well. |
| DNS (dinitrosalicylicacid ) Reagent . | -Solution “A” Weight carefully 6.25 grams of DNS , and 20 grams of NaOH , then dissolve in a small volume of water (to dissolve heat on a hot plate ), transfer to a 500 ml volumetric flask and add water up to the 500ml mark , mix well.-Solution “B” Weight carefully 300 grams of Putasium Sodium Tartarrate , dissolve in a small volume of water , transfer to a 500 ml volumetric flask then make up to 500ml by adding water , mix well .To Prepare the DNS Reagent : Take 222.2 ml of solution “A” add to it 277.7 ml of solution “B” mix well, (heat on a hot plate when needed).  |
| 0.1M Sodium Bicarbonate  | Weight carefully 0.840 gram of sodium bicarbonate dissolve in a small volume of water, transfer to a 100ml volumetric flask, make up the volume to 100ml by adding water , mix well . |
| Beta Fructofuranosidase(Invertase ) enzyme extract from yeast. | Weight carefully 10grams of dry yeast dissolve in 30 ml of a 0.1M sodium bicarbonate solution , mix by magnetic stirrer for 2 hours at room temperature , then centrifuge at 3000 rpm for 10 min , collect the supernatant carefully and label as enzyme extract .  |

Diluted Enzyme Preparation table (1):

|  |  |  |  |
| --- | --- | --- | --- |
| Enzyme solution | Dilution | Enzyme concentration x10-3 | Enzyme dilution preparation |
| A | 1:125 |  8.0X | 1.6ml enzyme extract and make up to 200ml by adding water. |
| B | 1:100 | 10X | 2.0ml enzyme extract and make up to 200ml by adding water. |
| C | 1: 66 |  15X | 3.0ml enzyme extract and make up to 200ml by adding water. |
| D | 1:50 | 20X | 4.0ml enzyme extract and make up to 200ml by adding water. |
| E | 1:33 | 30X |  6.0ml enzyme extract and make up to 200ml by adding water. |
| F | 1: 16 | 60X | 12.0ml enzyme extract and make up to 200ml by adding water. |

**Method:**

1- Prepare 8 test tubes in the following manner table (2):

|  |  |  |
| --- | --- | --- |
| Tube | Acetate buffer (ml) |  0.18M Sucrose (ml) |
| Blank | 1.0 | 2.0  |
| A | 1.0 | 2.0  |
| B | 1.0 | 2.0  |
| C | 1.0 | 2.0  |
| D | 1.0 | 2.0  |
| E | 1.0 | 2.0  |
| F | 1.0 | 2.0  |

2- Mix each tube then add 0.05ml of diluted enzyme according to the following table (2) , EXCEPT FOR THE BLANK ADD 0.05ml OF DISTILLED WATER INSTEAD , mix and start the stop clock immediately , incubate each tube for 10 min , then stop the reaction by adding 2.0ml of the DNS reagent to each tube.

Note: Mix each tube frequently during the incubation time.

Table (3)

|  |  |  |
| --- | --- | --- |
| Tube | Enzyme Solution | Enzyme concentration x 10-3 |
| Blank | --- | 0 |
| A | E1 | 8.0X |
| B | E2 |  10.0X |
| C | E3 |  15.0X |
| D | E4 | 20X |
| E | E5 | 30X |
| F | E6 | 60X |

Table (4)

|  |  |  |
| --- | --- | --- |
| Tube | Start (min) | Stop (min) |
| Blank | 0.0 | 0.0 |
| A | 1.0 | 11.0 |
| B | 2.0 | 12.0 |
| C | 3.0 | 13.0 |
| D | 4.0 | 14.0 |
| E | 5.0 | 15.0 |
| F | 6.0 | 16.0 |

3- Mix properly, cover each tube by aluminium foil and place in a boiling water bath for 5min to allow the colour to develop.

4- Then remove from water bath cool under tap water, add 20ml of distilled water to each tube, mix properly then measure the absorbance at 540nm.

5- Record the absorbance of each test tube in the following table (4),

6- Convert the Absorbance reading obtained to micromoles of sucrose hydrolyzed making use of the standard reducing sugars calibration curve , then divide by 10 to obtain the number of micromoles of sucrose hydrolyzed /min (vi ).

7 – Draw a graph between the micromoles of sucrose hydrolyzed /min (vi ) and enzyme concentration .

Table (5)

|  |  |  |  |
| --- | --- | --- | --- |
| Tube | Absorbance 540nm | µmoles of sucrose hydrolyzed | µmoles /min( vi )  |
| A | 0.116  | 900 | 90 |
| B |  0.177 | 1350 | 135 |
| C |  0.227 | 1750 | 175 |
| D |  0.258 | 2000 | 200 |
| E |  0.379 | 3000 | 300 |
| F |  0.695 | --- | --- |

**Graph for Effect of Enzyme Concentration on Enzyme Activity**

 

**Experiment No.4**

**Effect of Temperature on the Rate of an Enzymatic Reaction**

 **Objective:**

1-To establish the relationship between temperature and the rate of an enzyme catalyzed reaction.

2-To determine the optimum temperature for β-Fructofuranosidase enzyme

**Material:**

 - 0.05M Sodium acetate buffer, pH 4.7

 - 0.3 M Sucrose

 -DNS (dinitrosalicylicacid ) Reagent

 -Sodium Bicarbonate

 - Beta Fructofuranosidase(Invertase ) enzyme extract from yeast

 -0.1M Sodium Bicarbonate

 -Yeast

**Glassware and Instrumentation:**

\_ Test tubes

- Pipettes

-Measuring cylinder (20ml).

- Water baths (4°C , 20°C, 27°C ,40°C , 50°C , 60°C, 100°C )

-Spectrophotometer

-Aluminium foil.

**Preparation of Solutions:**

|  |  |
| --- | --- |
| **Solution** | **Preparation** |
|  0.05M SodiumAcetate buffer , pH 4.7 .  | Weight carefully 1.903g of sodium acetate (CH3COONa) , dissolve in a small volume of distilled water , then add to it 1.5ml of 99.7% Glacial Acetic acid , transfer all to a 1L volumetric flask then make up the volume to 1L by adding distilled water. Then check pH , and adjust if necessary . |
| 0.3 M Sucrose . | Weight carefully 102.69 grams of sucrose dissolve in a small volume of water , transfer to a 1L volumetric flask , then make up to 1L by adding water , mix well . |
| DNS (dinitrosalicylicacid ) Reagent . | -Solution “A” Weight carefully 6.25 grams of DNS , and 20 grams of NaOH , then dissolve in a small volume of water (to dissolve heat on a hot plate ), transfer to a 500 ml volumetric flask and add water up to the 500ml mark , mix well.-Solution “B” Weight carefully 300 grams of Putasium Sodium Tartarrate , dissolve in a small volume of water , transfer to a 500 ml volumetric flask then make up to 500ml by adding water , mix well .To Prepare the DNS Reagent : Take 222.2 ml of solution “A” add to it 277.7 ml of solution “B” mix well , (heat on a hot plate when needed ).  |
| 0.1M Sodium Bicarbonate . | Weight carefully 0.840 gram of sodium bicarbonate dissolve in a small volume of water , transfer to a 100ml volumetric flask, make up the volume to 100ml by adding water , mix well . |
| Beta Fructofuranosidase(Invertase ) enzyme extract from yeast. | Weight carefully 10grams of dry yeast dissolve in 30 ml of a 0.1M sodium bicarbonate solution , mix by magnetic stirrer for 2 hours at room temperature , then centrifuge at 3000 rpm for 10 min , collect the supernatant carefully and label as enzyme extract . Just before start of experiment prepare a diluted enzyme extract solution 1:200 by taking 1.0ml of concentrated enzyme extract and adding water up to 200ml in a volumetric flask mix gently  |

**Method:**

1- Prepare 14 test tubes in the following manner, table (1):

|  |  |  |
| --- | --- | --- |
| Tube | Acetate buffer (ml) |  0.3M Sucrose (ml) |
| ABlank A  | 1.0 | 2.0  |
| 1.0 | 2.0  |
| B Blank B | 1.0 | 2.0  |
| 1.0 | 2.0  |
| CBlank C | 1.0 | 2.0  |
| 1.0 | 2.0  |
| DBlank D | 1.0 | 2.0 |
| 1.0 | 2.0 |
| EBlank E | 1.0 | 2.0 |
| 1.0 | 2.0 |
| FBlank F | 1.0 | 2.0 |
| 1.0 | 2.0 |
| G | 1.0 | 2.0 |
| Blank G | 1.0 | 2.0 |

2- Mix, then place each tube and its corresponding blank in the corresponding water bath and leave for 5 min to reach the required temperature

Table (2)

|  |  |
| --- | --- |
| Tube | Temperature  |
|  A |  4˚C |
|  B | 20 ˚C  |
| C |  27˚C  |
| D | 40˚C |
| E |  50˚C |
| F | 60˚C |
|  G | 100 |

3- Then add 0.05ml of distilled water to the Blank tubes and 0.05ml of the enzyme to the test tubes according to the following time table (3), mix and incubate all tubes for 10 minutes. Stop the reaction by adding 2ml of DNS to all tubes according to the timing in table (3).

**Note:** Mix each tube frequently during the incubation time

Table (3)

|  |  |  |
| --- | --- | --- |
| Tube | Start | Stop |
| Blank AA | 0 | 10  |
| 1.0 |  11 |
| Blank BB | 2.0 |  12 |
| 3.0 | 13  |
| Blank CC | 4.0 | 14  |
| 5.0 | 15  |
| Blank DD | 6.0 | 16 |
| 7.0 | 17 |
| Blank EE | 8.0 | 18 |
| 9.0 | 19 |
| Blank FF | 10.0 | 20 |
| 11.0 | 21 |
| Blank G | 12 | 22 |
| G | 13 | 23 |

4- Mix properly, remove from water bath, cover each tube by aluminium foil and place in a boiling water bath for 5min to allow the colour to develop .

5- Then remove from boiling water bath, cool under tap water , add 20ml of distilled water to each tube , mix properly then measure the absorbance at 540nm against corresponding blank..

6- Record the absorbance of each test tube in the following table ( 4),

7- Convert the Absorbance reading obtained to micromoles of sucrose hydrolyzed making use of the standard reducing sugars calibration curve , then divide by 10 to obtain the number of micromoles of sucrose hydrolyzed /min (vi ).

8 – Draw a graph between the micromoles of sucrose hydrolyzed /min (vi ) and Temperature .

Table (4)

|  |  |  |  |
| --- | --- | --- | --- |
| Tube | Absorbance 540nm | µmoles of sucrose hydrolyzed | µmoles /min( vi ) |
| A | 0.024 | 200 | 20 |
| B | 0.048 | 400 | 40 |
| C | 0.072 | 600 | 60 |
| D | 0.131 | 1100 | 110 |
| E | 0.202 | 1650 | 165 |
| F | 0.298 | 2400 | 240 |
| G | 0.106 | 800 | 80 |

**Graph of the Effect of Temperature on the Rate of an Enzymatic Reaction**

**Experiment No. 5**

**Effect Of pH on The Rate of an Enzymatic Reaction**

**Objective**:

1-To establish the relationship between pH and the rate of an enzyme catalyzed reaction.

2-To determine the optimum pH for β-Fructofuranosidase enzyme.

**Material:**

-0.05M Sodium Acetate buffer pH 4.7.

-0.05M phosphate buffer pH3.0.

- 0.05M phosphate buffer pH 7.0.

-0.05M phosphate buffer pH 8.0.

- Buffer pH 10.0.

-0.25 M Sucrose solution

-DNS (dinitrosalicylicacid ) Reagent - Beta Fructofuranosidase(Invertase ) enzyme extract from yeast.

-0.1M Sodium Bicarbonate

-Yeast

**Glassware and Instrumentation:**

\_ Test tubes

- Pipettes

-Measuring cylinder (20ml)

- Water baths (40°, 100°)

-Spectrophotometer

-Aluminium foil

**Preparation Of solutions:**

|  |  |
| --- | --- |
| **Solution** | **Preparation** |
|  Preparation of buffers :1-0.1 L of 0.05M H3PO4 /KH2PO4 buffer , pH 3.0 .2- 1L of 0.05M Sodium Acetate buffer , pH 4.7 . 3-0.1 L of 0.05M KH2PO4/ K2HPO4 buffer , pH 7.0 .4- 0.1L of 0.05M  KH2PO4/ KH2PO4 buffer , pH 8.0 . 5- 0.1L of 0.05M KH2PO4/K3PO4, pH 12.0. | Weight carefully 0.598g of KH2PO4 , dissolve in a small volume of distilled water , then add to it 0.04 ml of 85% Phosphoric acid , transfer all to a 0.1L volumetric flask , make up the volume to 0.1L by adding distilled water. Then check pH , and adjust if necessary .Weight carefully 1.903g of sodium acetate (CH3COONa) , dissolve in a small volume of distilled water , then add to it 1.5ml of 99.7% Glacial Acetic acid , transfer all to a 1L volumetric flask then make up the volume to 1L by adding distilled water. Then check pH , and adjust if necessary .Weight carefully 0.3362g of K2HPO4 , and 0.417g of KH2PO4  dissolve in a small volume of distilled water , , transfer all to a 0.1L volumetric flask then make up the volume to 0.1L by adding distilled water. Then check pH , and adjust if necessary . Weight carefully 0.749g of K2HPO4 , and 0.0952g of KH2PO4  dissolve in a small volume of distilled water , , transfer all to a 0.1L volumetric flask then make up the volume to 0.1L by adding distilled water. Then check pH , and adjust if necessary . Weight carefully 0.352g of K3PO4 , and 0.5818g of KH2PO4 dissolve in a small volume of distilled water , , transfer all to a 0.1L volumetric flask then make up the volume to 0.1L by adding distilled water. Then check pH , and adjust if necessary . |
| 0.25 M Sucrose . volume 250ml . | Weight carefully 21.39 grams of sucrose dissolve in a small volume of water , transfer to a 250ml volumetric flask , then make up to 250ml by adding water , mix well . |
| DNS (dinitrosalicylicacid ) Reagent . | -Solution “A” Weight carefully 6.25 grams of DNS , and 20 grams of NaOH , then dissolve in a small volume of water (to dissolve heat on a hot plate ), transfer to a 500 ml volumetric flask and add water up to the 500ml mark , mix well.-Solution “B” Weight carefully 300 grams of Putasium Sodium Tartarrate , dissolve in a small volume of water , transfer to a 500 ml volumetric flask then make up to 500ml by adding water , mix well .To Prepare the DNS Reagent : Take 222.2 ml of solution “A” add to it 277.7 ml of solution “B” mix well , (heat on a hot plate when needed ).  |
| 0.1M Sodium Bicarbonate . | Weight carefully 0.840 gram of sodium bicarbonate dissolve in a small volume of water , transfer to a 100ml volumetric flask, make up the volume to 100ml by adding water , mix well . |
| Beta Fructofuranosidase(Invertase ) enzyme extract from yeast. | Weight carefully 10grams of dry yeast dissolve in 30 ml of a 0.1M sodium bicarbonate solution , mix by magnetic stirrer for 2 hours at room temperature , then centrifuge at 3000 rpm for 10 min , collect the supernatant carefully and label as enzyme extract . Just before start of experiment prepare a diluted enzyme extract solution 1:200 by taking 1.0ml of concentrated enzyme extract and adding water up to 200ml in a volumetric flask mix gently . |

Method:

1. Prepare 6 test tubes of different pH values by following the table (1)provided : Note due to lack of chemical components a buffer of pH 10 was used instead of buffer pH 12.0 in this experiment .

|  |  |  |
| --- | --- | --- |
| Tube |  Buffer 1.0 (ml) |  Sucrose (ml) |
| A | pH 3.0  | 2.0  |
| Blank A | pH 3.0  | 2.0  |
| B | pH 4.7  | 2.0  |
| Blank B | pH 4.7  |  2.0 |
| C |  pH 7.0  |  2.0 |
| Blank C | pH 7.0  |  2.0 |
| D | pH 8.0 | 2.0 |
| Blank D | pH 8.0 | 2.0 |
| E | pH 10.0 | 2.0 |
| Blank E | pH10.0 | 2.0 |

2- Mix each tube properly then incubate all tubes at 40˚C for 5min

3- Start the reaction by adding 0.05ml of diluted enzyme to all test tubes tubes except for the blanks add 0.05ml of distilled water instead , mix and start the stop clock immediately , incubate each tube for 10min , then stop the reaction by adding 2.0ml of the DNS reagent to each tube and mix well .(follow table 2 for adding enzyme and DNS to tubes ) .

**Note:** Mix each tube frequently during the incubation time

Table (2)

|  |  |  |
| --- | --- | --- |
| Tube | Start Time (min) | Stop by adding 2.0ml DNS (min) |
| Blank A | 0 | 10 |
| Blank B | 1.0 | 11 |
| Blank C | 2.0 | 12 |
| Blank D | 3.0 | 13 |
| Blank E | 4.0 | 14 |
| A | 5.0 | 15 |
|  |  |  |
|  |  |  |
| B | 6.0 | 16 |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
| C | 7.0 | 17 |
|  |  |  |
|  |  |  |
| D | 8.0 | 18 |
|  |  |  |
|  |  |  |
| E | 9.0 | 19 |
|  |  |  |
|  |  |  |

4- Mix properly, cover each tube by aluminium foil and place in a boiling water bath for 5min to allow the colour to develop

5- Remove from water bath cool under tap water, add 20ml of distilled water to each tube, mix properly then measure the absorbance at 540nm

6- Record the absorbance of each test tube in the following table (3),

7- Convert the absorbance reading obtained to micromoles of sucrose hydrolyzed making use of the standard reducing sugars calibration curve , determine the initial velocity vi  for each tube and record all in table 3 .

8- Obtain the relationship between the initial velocity vi  and pH , by drawing a graph between the initial velocity vi  and pH . Determine the optimum pH for your enzymatic reaction.

Table (3)

|  |  |  |  |
| --- | --- | --- | --- |
| Tube | Absorbance 540nm | µmoles of sucrose hydrolyzed | µmoles of sucrose hydrolyzed/min(vi) |
| A | 0.178  | 250  | 25 |
| B | 0.528 |  750 | 75 |
| C |  0.123 |  150 | 15 |
| D | 0.103  |  100 | 10 |
| E | 0.021  |  25 | 2.5 |

**Graph of effect of pH on the rate of an enzymatic reaction**

**Experiment No. 6**

 **Effect of Substrate Concentration on the Rate of an Enzymatic Reaction**

**Objective:**

1-To establish the relationship between substrate concentration and the rate of an enzyme catalyzed reaction.

2-To determine the Km and V max of the enzyme for a particular substrate.

**Material:**

**-**0.05M Sodium Acetate buffer, pH 4.7

**-**0.25 M Sucrose stock solution

-DNS (dinitrosalicylicacid ) Reagent

- Beta Fructofuranosidase(Invertase ) enzyme extract from yeast.

 -0.1M Sodium Bicarbonate

 - Yeast

**Glassware and Instrumentation:**

\_ Test tubes

- Pipettes

-Measuring cylinder (20ml).

- Water baths (40°, 100° )

-Spectrophotometer

- Aluminium foil

**Preparation Of solutions:**

|  |  |
| --- | --- |
| **Solution** | **Preparation** |
|  0.05M Sodium Acetate buffer , pH 4.7 .  | Weight carefully 1.903g of sodium acetate (CH3COONa) , dissolve in a small volume of distilled water , then add to it 1.5ml of 99.7% Glacial Acetic acid , transfer all to a 1L volumetric flask then make up the volume to 1L by adding distilled water. Then check pH , and adjust if necessary . |
| 0.25 M Sucrose . volume 250ml . | Weight carefully 21.3 grams of sucrose dissolve in a small volume of water , transfer to a 250ml volumetric flask , then make up to 250ml by adding water , mix well . |
| DNS (dinitrosalicylicacid ) Reagent . | -Solution “A” Weight carefully 6.25 grams of DNS , and 20 grams of NaOH , then dissolve in a small volume of water (to dissolve heat on a hot plate ), transfer to a 500 ml volumetric flask and add water up to the 500ml mark , mix well.-Solution “B” Weight carefully 300 grams of Putasium Sodium Tartarrate , dissolve in a small volume of water , transfer to a 500 ml volumetric flask then make up to 500ml by adding water , mix well .To Prepare the DNS Reagent : Take 222.2 ml of solution “A” add to it 277.7 ml of solution “B” mix well , (heat on a hot plate when needed ).  |
| 0.1M Sodium Bicarbonate . | Weight carefully 0.840 gram of sodium bicarbonate dissolve in a small volume of water , transfer to a 100ml volumetric flask, make up the volume to 100ml by adding water , mix well . |
| Beta Fructofuranosidase(Invertase ) enzyme extract from yeast. | Weight carefully 10grams of dry yeast dissolve in 30 ml of a 0.1M sodium bicarbonate solution , mix by magnetic stirrer for 2 hours at room temperature , then centrifuge at 3000 rpm for 10 min , collect the supernatant carefully and label as enzyme extract . Just before start of experiment prepare a diluted enzyme extract solution 1:200 by taking 1.0ml of concentrated enzyme extract and adding water up to 200ml in a volumetric flask mix gently |

**Method:**

1. Prepare 7 test tubes of different substrate (sucrose) concentrations by following the table provided. ( 1 ) :

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tube | Distilled water (ml) |  0.25M Sucrose (ml) | Concentration of sucrose M | Actual \*Concentration of sucrose M |
| Blank |  2.0 |  0.0 | 0.0 | 0.0 |
| A |  1.8 | 0.2  | 0.025 | 0.016 |
| B |  1.6 | 0.4 | 0.05 | 0.032 |
| C |  1.2 | 0.8 | 0.1 | 0.0655 |
| D |  1.0 | 1.0 | 0.125 | 0.081 |
| E |  0.5 | 1.5 | 0.1875 | 0.122 |
| F |  0.0 | 2.0 | 0.25 | 0.163 |

Note the actual concentration of sucrose \* is the accurate substrate concentration accounting for the diluting effect of both the acetate buffer and the enzyme, sucrose has been diluted by a factor of 0.655 in each tube .

 2-Mix each tube properly then add 1.0ml of the Acetate buffer to each tube mix well

 3-Incubate all tubes at 40˚C for 5min

4- Start the reaction by adding 0.05ml of diluted enzyme to all tubes , mix and start the stop clock immediately , incubate each tube for 10min , then stop the reaction by adding 2.0ml of the DNS reagent to each tube and mix well .(follow table 2 for adding enzyme and DNS to tubes ) .

**Note:** Mix each tube frequently during the incubation time

Table (2)

|  |  |  |
| --- | --- | --- |
| Tube | Start Time (min) | Stop by adding 2.0ml DNS . (min) |
| Blank  | 0 | 10 |
|  |  |  |
| A | 1.0 | 11 |
| B | 2.0 | 12 |
| C | 3.0 | 13 |
| D | 4.0 | 14 |
| E | 5.0 | 15 |
| F | 6.0 | 16 |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |

5- Mix properly, cover each tube by aluminium foil and place in a boiling water bath for 5min to allow the colour to develop

6- Remove from water bath cool under tap water, add 20ml of distilled water to each tube , mix properly then measure the absorbance at 540nm .

6- Record the absorbance of each test tube in the following table (3).

7- Convert the Absorbance reading obtained to micromoles of sucrose hydrolyzed making use of the standard reducing sugars calibration curve , determine the initial velocity vi  for each tube and record all in table 3 .

8- Draw a graph of vi vs [S] (Michealis curve) , and a graph between 1/vi  on the y-axis ans 1/[S] on the x-axis(linweaver and Burk Plot ) . Determine the Vmax and Km for the enzyme /substrate pair you are studying.

Note the concentration of sucrose \* is the accurate substrate concentration accounting for the diluting effect of both the acetate buffer and the enzyme , sucrose has been diluted by a factor of 0.655 in each tube .

Table (3)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tube | Absorbance 540nm | Concentration of sucrose \*[S] M | 1/[S] | µmoles of sucrose hydrolyzed | µmoles of sucrose hydrolyzed/min(vi) | 1/vi |
| A | 0.163 | 0.016 | 62.5 | 250 | 25 | 0.04 |
| B | 0.319 | 0.032 | 31.25 | 450 | 45 | 0.022 |
| C | 0.422 | 0.0655 | 15.26 | 600 | 60 | 0.0166 |
| D | 0.622 | 0.081 | 12.34 | 900 | 90 | 0.011 |
| E | 0.512 | 0.122 | 8.19 | 700 | 70 | 0.014 |
| F | 0.821 | 0.163 | 6.13 | 1150 | 115 | .00869 |

Michealis -Menten Graph



**Experiment No. 7**

 **Enzyme inhibition**

**Objective:**

1. To determine the type of inhibition of invertase by copper sulfate

**Material:**

-0.05M Sodium acetate buffer, pH 4.7

-0.25 M Sucrose stock solution

-0.018M copper sulfate

 -DNS (dinitrosalicylicacid) Reagent -Beta Fructofuranosidase(Invertase ) enzyme extract from yeast

-0.1M Sodium Bicarbonate

- Yeast

**Glassware and Instrumentation:**

\_ Test tubes

- Pipettes

-Measuring cylinder (20ml)

- Water baths (40°, 100°)

-Spectrophotometer

-Aluminium foil.

**Preparation Of solutions:**

|  |  |
| --- | --- |
| **Solution** | **Preparation** |
|  0.05M Sodium Acetate buffer , pH 4.7 .  | Weight carefully 1.903g of sodium acetate (CH3COONa) , dissolve in a small volume of distilled water , then add to it 1.5ml of 99.7% Glacial Acetic acid , transfer all to a 1L volumetric flask then make up the volume to 1L by adding distilled water. Then check pH , and adjust if necessary . |
| 0.25 M Sucrose . volume 250ml . | Weight carefully 21.3 grams of sucrose dissolve in a small volume of water , transfer to a 250ml volumetric flask , then make up to 250ml by adding water , mix well . |
| DNS (dinitrosalicylicacid ) Reagent . | -Solution “A” Weight carefully 6.25 grams of DNS , and 20 grams of NaOH , then dissolve in a small volume of water (to dissolve heat on a hot plate ), transfer to a 500 ml volumetric flask and add water up to the 500ml mark , mix well.-Solution “B” Weight carefully 300 grams of Putasium Sodium Tartarrate , dissolve in a small volume of water , transfer to a 500 ml volumetric flask then make up to 500ml by adding water , mix well .To Prepare the DNS Reagent : Take 222.2 ml of solution “A” add to it 277.7 ml of solution “B” mix well , (heat on a hot plate when needed ).  |
| 0.1M Sodium Bicarbonate . | Weight carefully 0.840 gram of sodium bicarbonate dissolve in a small volume of water , transfer to a 100ml volumetric flask, make up the volume to 100ml by adding water , mix well . |
| Beta Fructofuranosidase(Invertase ) enzyme extract from yeast. | Weight carefully 10grams of dry yeast dissolve in 30 ml of a 0.1M sodium bicarbonate solution , mix by magnetic stirrer for 2 hours at room temperature , then centrifuge at 3000 rpm for 10 min , collect the supernatant carefully and label as enzyme extract . Just before start of experiment prepare a diluted enzyme extract solution 1:200 by taking 1.0ml of concentrated enzyme extract and adding water up to 200ml in a volumetric flask mix gentlymark by adding acetate buffer (pH 4.7 and 0.05M ). |
| -0.018M copper sulfate . | First prepare 0.1M copper sulfate solution ; Weight carefully0.798g of anhydrous copper sulfate dissolve in a total volume of 50ml of acetate buffer( (pH 4.7 and 0.05M ) , then take 45ml of the 0.1M solution copper sulfate prepared and transfer to a 250ml volumetric flak then make up to the mark by adding distilled water. |

**Method:**

1. Prepare two sets of test tubes SET 1 WITHOUT INHIBITOR :

Prepare 7 test tubes of different substrate (sucrose) concentrations by following the table provided. (1)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tube | Distilled water (ml) |  0.25M Sucrose (ml) | Concentration of sucrose M | Actual Concentration of sucrose M |
| Blank |  2.0 |  0.0 | 0.0 | 0.0 |
| A |  1.8 | 0.2  | 0.025 | 0.016 |
| B |  1.6 | 0.4 | 0.05 | 0.032 |
| C |  1.2 | 0.8 | 0.1 | 0.0655 |
| D |  1.0 | 1.0 | 0.125 | 0.081 |
| E |  0.5 | 1.5 | 0.1875 | 0.122 |
| F |  0.0 | 2.0 | 0.25 | 0.163 |

1. Mix each tube properly then add 1.0ml of the Acetate buffer to each tube mix well .
2. Prepare SET2 WITH INHIBITOR table (2) :

|  |  |  |  |
| --- | --- | --- | --- |
| Tube | Distilled water (ml) |  0.25M Sucrose (ml) | 0.018M Copper sulphate (ml) |
| Blank Inhibitor |  2.0 |  0.0 | 1.0 |
| G  |  1.8 | 0.2  | 1.0 |
|  H |  1.6 | 0.4 | 1.0 |
|  I |  1.2 | 0.8 | 1.0 |
| J  |  1.0 | 1.0 | 1.0 |
| K  |  0.5 | 1.5 | 1.0 |
|  L |  0.0 | 2.0 | 1.0 |

 4- Mix well then , incubate all tubes at 40˚C for 5min .

5- Start the reaction by adding 0.05ml of diluted enzyme to all tubes , mix and start the stop clock immediately , incubate each tube for 10min , then stop the reaction by adding 2.0ml of the DNS reagent to each tube and mix well .(follow table 3 for adding enzyme and DNS to tubes ) .

**Note**: Mix each tube frequently during the incubation time

Table (3)

|  |  |  |
| --- | --- | --- |
| Tube | Start Time (min) | Stop by adding 2.0ml DNS (min) |
| Blank  | 0 | 10 |
|  |  |  |
| A | 1.0 | 11 |
| B | 2.0 | 12 |
| C | 3.0 | 13 |
| D | 4.0 | 14 |
| E | 5.0 | 15 |
| F | 6.0 | 16 |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
| Blank Inhibitor | 7.0 | 17 |
| G | 8.0 | 18 |
| H | 9.0 | 19 |
| I |  10 | 20 |
| J | 11 | 21 |
| K | 12 | 22 |
|  L | 13 | 23 |

4- Mix properly, cover each tube by aluminium foil and place in a boiling water bath for 5min to allow the colour to develop.

5- Remove from water bath cool under tap water, add 20ml of distilled water to each tube , mix properly then measure the absorbance at 540nm .

6- Record the absorbance of each test tube in the following table (4),(5).

7- Convert the Absorbance reading obtained to micromoles of sucrose hydrolyzed making use of the standard reducing sugars calibration curve , determine the initial velocity vi  for each tube and record all in table 4 .

8- Draw a graph between 1/vi  on the y-axis and 1/[S] on the x-axis, for both sets of reactions, with Inhibitor and without inhibitor (linweaver and Burk Plot) and determine the type of inhibition of the copper sulfate inhibitor

Note the concentration of sucrose \* is the accurate substrate concentration accounting for the diluting effect of both the acetate buffer and the enzyme, sucrose has been diluted by a factor of 0.655 in each tube

Table (4) Without Inhibitor

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tube | Absorbance 540nm | Concentration of sucrose \* | 1/[S] | µmoles of sucrose hydrolyzed | µmoles of sucrose hydrolyzed/min(vi) | 1/vi |
| A | 0.038  | 0.016 | 62.5 | 70  | 7.0  | 0.14  |
| B |  0.208 | 0.032 | 31.25 |  300 | 30  | 0.03  |
| C | 0.225  | 0.0655 | 15.26 |  350 | 35  | 0.028  |
| D | 0.283  | 0.081 | 12.34 |  400 | 40  | 0.025  |
| E | 0.319  | 0.122 | 8.19 |  450 | 45  | 0.022  |
| F | 0.351  | 0.163 | 6.13 |  500 | 50  | 0.020  |

Table (5) With Inhibitor

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tube | Absorbance 540nm | Concentration of sucrose \* | 1/[S] | µmoles of sucrose hydrolyzed | µmoles of sucrose hydrolyzed/min(vi) | 1/vi |
| G | 0.008  | 0.016 | 62.5 |  18 | 1.8 | 0.55 |
| H | 0.011  | 0.032 | 31.25 | 25 | 2.5 | 0.4 |
| I | 0.028  | 0.0655 | 15.26 |  50 | 5.0 | 0.2 |
| J | 0.047  | 0.081 | 12.34 |  75 | 7.5 | 0.133 |
| K | 0.079  | 0.122 | 8.19 |  125 | 12.5 | 0.08 |
| L | 0.130  | 0.163 | 6.13 |  200 | 20 | 0.05 |

**Linweaver and Burk Plot**



**Experiment No.8**

**Methods of Enzyme Assay**

**Objective:**

Study the Continuous assay method by determining the enzymes activity for:

1. Alanine transaminase (ALT)

2. Lactate dehydrogenase (LDH)

**Material:**

-ALT Kit

-LDH Kit

-Normal Serum kit

-Abnormal serum kit

**Glassware and Instrumentation:**

\_Test tubes

- Pipettes

- Water baths (37°)

- Quartz cuvette

-Spectrophotometer

-Stop watch

|  |
| --- |
| **T1**  |
| Pipette **3ml** of the ALT reagent  |
| Pre-warm the tubes at **37 for 3 min**  |
| Pipette **0.2ml /200µl**  of serum sample  |
| Mix , and allow 60 seconds for temperature equilibration  |
| Read the absorbance at **340nm** every minute for 3 minute /use(H2O) as blank  |

**Method**

|  |
| --- |
| **T1**  |
| Pipette **3ml** of the LDH reagent  |
| Pre-warm the tubes at **37 for 3 min**  |
| Pipette **0.1 ml/100µl** of serum sample  |
| Mix , and allow 60 seconds for temperature equilibration  |
| Read the absorbance at **340nm every minute for 3** minute /use(H2O) as blank  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Absorbance 1min | Absorbance 2min | Absorbance 3min |
| Normal serum | 1.560 | 1.546 | 1.528 |
| Abnormal serum | 2.087 | 2.054 | 2.021 |

* **Determination of ALT in serum:**

**For normal serum**

ΔA/min x 1768=

ΔA/ min= (1.560- 1.546) + (1.546- 1.528) / 2=

=0.016 X 1768= 28.3 U/L

**For abnormal serum**

ΔA/min x 1768=

ΔA/ min= (2.087-2.054) + (2.054-2.021)/ 2=

= 0.033 X 1768= 58.3 U/L

The normal range up to 32 U/L

* **Determination of LDH in serum:**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Ab1 after 1 min. | Ab2 after 2min | Ab3 after 3 min. |
| Normal serum | 0.476 | 0.500 | 0.514 |

**LDH activity U/L**

ΔA/ min (normal serum) = (0.500-0.476) +(0.514-0.500) /2

=0.019 X4984 =**94.6 U/L**

**The normal range = 103 – 227 U/L**