**Objectives**

The aim of this experiment is to assess the lysosomal enzyme (acid phosphatase) in the isolated lysosomal fraction in Experiment No. 3.

It is the measurement of the reaction's color, The nitrophenyl phosphate substrate will hydrolyze to p-nitrophenol, a yellow substance.

When the enzyme is present (provided by the lysosomal fraction). Spectrophotometer is a tool that may be utilized.

To determine the existence of the enzyme by measuring color development.

**Introduction**

The smallest unit that can exist on its own is the cell. An organism is made up of all living entities and tissues.

The primary difference between prokaryotes and eukaryotic cells is that prokaryotes are smaller than eukaryotic cells.

Cell types are eukaryotic cells' membrane-bound nuclei.

In addition to the eukaryotic nucleus, cells may include a variety of additional organelles, the most significant of which are lysosomes in this experiment.

The lysosome is membrane bound after the cellular material has been digested by division or phagocytosis.

The organelle with the most digestive enzymes Acid phosphorylase, for example, is a good biomarker of enzymatic digestion in cells.In this work, we measured the absorption of p-nitrophenol in photometrically diluted solutions at 405 nm at different time intervals to quantify Acid phosphate reductase activity using the lysosomal fraction separated by differential centrifugation.

**Material**

1\_1M Sodium acetate buffer, pH= 5.7

2\_0.1 M MgCl2

3\_50 mM p-nitrophenyl phosphate (pNPP)

4\_Lysosomal Fraction (Tube L from experiment 4)

5\_Water bath at 37oC

6\_0.5 N KOH

7\_Spectrophotometer

8\_Tubes

9\_Distilled water

10\_Vortex Mixer

**Method**

1\_6 tubes were labeled: BLANK, T1, T2, T3, T4, T5, then the same amounts of

The following solutions were added to all tubes: 0.5 ml acetate, 0.5 ml MgCl2, 0.5 ml

pNPP, 3.3 ml H20.

2\_200 μl HEPES and 2 mL KOH were added to the empty tube. The solution was well vortexed, and used to discharge the spectrophotometer at 405 nm.

3\_To tube 1, 200 μL of the stock lysosomal fraction was added and vortexed well.

4\_Tagged 5 other test tubes: S1, S2, S3, S4, S5. Then, 1.8 mL of HEPES was added to each tube.

5\_To S1, 200 μL of the stock lysosomal fraction was added, and vortexed well.

6\_ A serial dilution was then performed on the remaining S-tubes, by taking 200 μl from the previous tube, and tipping well between dilutions.

7\_of the S-tubes, 200 μl was taken and added to T-tubes, and vortexed well

Then (example: from S1, S2, S3, S4, S5 to T1, T2, T3, T4, T5, respectively).

8\_ The T-tubes were placed in a hot bath at 37°C for exactly 5 minutes.

After the hot bath, 2 ml of KOH was added to each tube.

9\_ After 5 min of KOH addition, 5 tubes were absorbed at 405 nm

measured. Of the tube absorbing between (0.2-0.4) 3 more test tubes

They were prepared and named T10, T20, and T30, respectively.

10\_These three test tubes were prepared exactly as T1 in step 1 and 3.

11\_The test tubes T10, T20 and T30 were placed in the hot bath for 10, 20 and 30

min, respectively.

12\_ After the specified time to each tube, 2 ml of KOH was added.

13\_5 minutes after addition of KOH, the absorbance of p-nitrophenol at 405 nm was measured.

**Data And Results:**

The absorbance of tube 1 at different time intervals at 405 nm was obtained after running the experiment, as given in table 6.1.

**Table 6.1: absorbance of tube 1 at different time**

**intervals.**

|  |  |  |  |
| --- | --- | --- | --- |
| College | Time (minute) | blank | Undiluted  (400ml) |
|  |  |  |  |
| 1 | 1 | 0 | 0.39 |
| 2 | 2 | 0 | 0.42 |
| 3 | 3 | 0 | 0.48 |
| 4 | 4 | 0 | 0.52 |
| 5 | 5 | 0 | 0.542 |

**Discussion**

Table 6.1 shows that absorption rises with time, implying that a file will take longer to absorb.

With time, the concentration of p-nitrophenol rises.

The concentration of p-nitrophenol may be determined using the following equation:

(absorption \* 7) / (18.8 \* 106 \* 0.2) p-nitrophenol concentration

Due to dilution of lysosomes, absorption decreases with p-nitrophenol concentration; also, the first tube with stock lysosomal lysosomes was added to it, and absorption was highest.

Based on this, the enzyme activity was determined using the following equation:

Enzyme activity = (p-nitrophenol concentration in tube with greatest absorption (L stock portion tube) / 5 minutes) \* dilution factor (25).