Birzeit University

**Biology and Biochemistry Department**

**BIOL 244**

**Cell Biology lab**

**Experiment 6: Analysis of subcellular fraction III.**

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**Titles: Analysis of subcellular fraction III**

**Objectives: to learn about lysosomal fraction fractionated and use it by differential centrifugation, to measure the activity of the acid phosphatase enzyme by spectrophotometer.**

**Introduction:**

**According to the definition of Christian de Duve: Lysosomes are membrane-bounded organelle that contains corrosive hydrolases with different specificities and whose primary work is the intracellular digestion of macromolecules, Lysosomes encase a wide assortment of acid hydrolases, counting proteases (cathepsins), lipases, glucosidases, and nucleases Macromolecules and other substrates reach the lysosomes by differing instruments, Once interior the lysosomes, the sequestered materials are debased and their building squares are recycled. Lysosomes were to begin with recognized the Subcellular fractionation by de Duve when trying to discover the structure where glucose 6 phosphatase was localized". This was made by differential centrifugation of liver homogenates get a mitochondrial fraction, followed by a sucrose slope that isolated lysosomes from other cell.**

 **Later, as progress was made in elucidating the broad function of lysosomes, also coined the terms “endocytosis,” “phagocytosis,” and “autophagy” to designate pathways that bring substrates for digestion in lysosomes and, today, are active fields of research in cell biology.**

**Lysosomes contain numerous hydrolytic proteins, which have been appeared to process after wide assortment of proteins. Besides, these proteins are conveyed in all sorts f layers, and can be related with the particular alteration, preparing. And catabolism of film proteins A wide extend of hydrolytic chemicals with pH optima within the acid range are show within the I the lysosomal division. Broad explanatory fractionation studies have shown that there may be numerous shapes of these enzymes which they are not restricted exclusively to the lysosomal fraction". The nearness of lysosomes can be decided by measuring the acid phosphatase action, this enzyme is considered lysosomal marker, the response is colorimetric. Within the nearness of the enzyme provided by the lysosomal division, in alkaline conditions, ALP hydrolyzes p-nitrophenyl phosphate to p-nitrophenol and inorganic phosphate "the substrate, 50 mM p-nitrophenyl phosphate, it become p-nitrophenol and appear a yellow color; this indicates that the enzyme is working”**

**Materials and Methods:**

**1M Sodium acetate buffer, pH 5.7 \ Lysosome fraction \ 150 mM KCl in 10mM HEPES,PH7.4 \ Water bath at 30c \ 0.1 M MgCl2 \ 50 Mm p-Nitrophenyl phosphate \ 0.5 N KOH \ Spectrophotometer and tubes \ Biuret protein assay.**

**The 6 test tubes was prepared and a 0.5 ml of 1 M sodium acetate buffer, 0.5 ml of 0.1 M MgCl2 and 0.5 ml of 0.05 M p-nitrophenyl phosphate was added in it. In addition, 3.3 ml of distilled H2O was added in each tubes and the water bath was put it is 30c. Then, 200 µl of lysosome suspension (tube L) with 1.8 ml of 150 mM KCL and 10 mM HEPES buffer was added and mixed to make a serial dilution of lysosome fraction. in a new tube, 200 µl of the diluted suspension with 1.8 ml of buffer was added and mixed it and we was recorded two dilution reading, the undiluted lysosome suspension was added and the diluted fraction was labeled as 1/10, 1/100, 1/1000 and 1/10000. Then, 200 µl of buffer and 2 ml of KOH was added in tube 1 from step 1. The spectrophotometer was determined on 405nm and tube 1 was used like blank. After that, 200 µl of the undiluted the lysosome fraction in tube 2 was added and mixed and the water bath was put it is 30c for 5 min, 2 ml of KOH to tube 2 is added and mixed and the absorbance was read immediately, the absorbance reading should between 0.3 - 0.4, if not, steps 6 and 7 was repeated and in tube 3 (1/10) was started and completed to tube 6 (1/10000). Then we using the absorbance reading and notice change of absorbance (0.3-0.4) in 5 min, determine of actual rate of absorbance and measure the initial rate.**

**Results:**

Table 1: Data table

|  |  |  |  |
| --- | --- | --- | --- |
| (Tube)dilution  | Absorbance (at 5 min ) | [p-nitrophenol](µM) | Activity/min (µM/min) |
| (1) Blank | 0 | 0 | 0 |
| (2) 100 | 0.39 | 2.074\*10-8 | 4.14\*10-9 |
| (3) 10-1 | 0.42 | 2.234\*10-8 | 4.46\*10-9 |
| (4) 10-2 | 0.48 | 2.553\*10-8 | 5.10\*10-9 |
| (5) 10-3 | 0.52 | 2.765\*10-8 | 5.53\*10-9 |
| (6) 10-4 | 0.542 | 2.882\*10-8 | 5.76\*10-9 |

**Figure1: This figure shows there is a direct relationship between absorption and concentration. When the time is gradually increased, the absorption increases, also the concentration of p-nitrophenol increases, this indicates that the reaction occurred.**

**Discussion:**

**In this experiment the Purpose to use the lysosome fraction fractionated by differential centrifugation, to measure the activity of the acid phosphatase enzyme by spectrophotometer at 405 nm. We get lysosome fraction from previously experiment (#Experiment 3) and we make a dilution and we use a p-nitrophenyl as a substrate for the reaction (it become p-nitrophenol and appear a yellow color; this indicates that the enzyme is working), we calculate absorbance using a spectrophotometer at a wavelength of 405 nm for 5 minutes by Beer lambert equation: A=ε. b. c, as shown in Table 1. "This figure shows there is a direct relationship between absorption and concentration. When the time is gradually increased, the absorption increases, also the concentration of p-nitrophenol increases, this indicates that the reaction occurred."**

* **Why we add KOH?**

**The structure of lysosome is highly acidic and we need to dilute him so we use KOH because it is a base.**

**References:**

* <https://www.biologyonline.com/dictionary/lysosome>
* <https://www.yourarticlelibrary.com/zoology/cell/lysosome-occurrence-morphology-functions-and-origins/30583>
* <https://www.pnas.org/content/110/33/13234>

**Appendices**:

**A = εcl**

**A it’s the absorbance**

**Ε it’s the coefficient =18.8\*106**

**C it’s the concentration**

**L it’s the optical pathlength = 1cm**

**EX🡪 tube 2**

**C = 18.8\*106 \*1 /0.39**