

**Department of Biology and Biochemistry**

**CELL BIOLOGY**

**(BIOL 244)**

**Analysis of subcellular fractions 2**

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**Analysis of subcellular fraction**

* **Objectives:**

Mesurment and determain the DNA concentration in the liver fraction.

* **Introduction:**

The ability to study the structure and properties of distilled cellular components has been made possible thanks to subcellular fractionation. Subcellular fractionation, in particular, has aided in the definition of membrane boundaries and has become important for the creation of cell-free assays that recreate complex cellular processes ,When it comes to enrichment and study of intracellular organelles and low abundant multiprotein complexes, subcellular fractionation and proteomics are a perfect match. Subcellular fractionation is a versatile and adaptable method for reducing sample complexity, and it works well when combined with high-resolution 2D gel/mass spectrometry processing as well as gel-independent methods.

* **Materials**

|  |  |
| --- | --- |
| * Fresh rat liver * H & HB buffers * Homogenizer * Ice * Petri dishes * 8 Mm CaCl2 in H-buffer * 50 ml centrifuge tubes * Votrex * Centrifuge * Micropipettes and tips * Scalpels and scissors * 150 Mm KCl in 10 mM HEPES, PH 7.4 | * Spectrophotometer * Micropipettes * Aceto-orcein (2% solution) * The homogenate fraction, resuspend fraction, mitochondria fraction, and lysosomes fraction. |

* **Method**

For this exberemint was isolated The homogenate fraction, resuspend fraction, mitochondria fraction, and lysosomes fraction,from the rat liver by kiiled a rat and removed its liver and put it in the ice-cold, we was cut 1.2g from the liver,we added amount of HB-buffer to the liver(thats help to remove the free fatty and lipids) and chop it into small piceces with scalpels,then homogenized by Place the mixture in a homogenizer tube and homogenize with a motor homogenizer (up to 10-15 strokes) while keeping it chilled, then centrifuged the homogenate at 400xg for 5 minutes thats to remove the cell debris and unbroken cells, after that in the centrufuge tubes appeared pellet( un broken cell ) we got rid of it, and the supernatant(SN1) thats what we need this is the whole homogenate, we collected it volume **(v\*)** and talked 2ml and saved it on ice on tube H; to use it later, we repeat the centerfuge for the rest of the homogenate(SN1) for 15 minute at 4000xg, in the senterfuge tube we had a pellet (nuclei) we resupended it in the same volume of **v\*** of H-buffer and saved it on ice in tube N, and supernatant(SN2) we plased into a clean centerfuge tube to sentrifuge it at 12000xg for 15 minute the pellet which resulted is the mitochondrial fraction we was resuspend it in the same volume of **v\*** of H-buffer and saved it on ice in tube M, and the supernatant(SN3) recorded its volume, to (SN3) we added 80 mMCaCl2 to yield final concentration of 8mM of CaCl 2 in the **sample 1 ml of CaCl per 9 ml of supernatant**, stirred gently and centrifuged at 20000xg for 25 minute at 4 C , this give a microsomal fraction enriched in lysosomes we resuspend the pellet in the same volume of **v\***of 150 mM KCL in 10 mM HEPES buffer, PH7 saved it on ice in tube L,we removed the supernatant, to determination the DNA concentration we was prepared 18 tube for the whole fraction ,6 tubes labeling for Homogenate , 6 tubes for Mitochondrial fraction, and 6 tubes for Nuclear fraction, tube number 1 from all was the stock tjat we was used to do serial dilution of each fraction by increased ratio by 5 as how it shwos in table 1 when do that we mixed the fraction well, the spectrophotometer was turned on and the wavelenght was set at **A260** to measure the obsorbance for all 24 tubes , for the absorbance between 1.5 and 2 we was re measured it at **A280** to determine the dilution factor for it by used this law :

**DNA concentration (µg/ml)=A260\*50 µg/ml\*dilution factor**

**Table 1. Serial dilutions for each fraction**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tube** | **0.25M**  **Sucrose(ml)** | **Previous tube (ml)** | **Vfinal (ml)** | **Final dilutionratio** |
| **1** |  | 1.25ml stocksolution |  |  |
| **2** | 1 | 0.25 | 1.25 | 1:5 |
| **3** | 1 | 0.25 | 1.25 | 1:25 |
| **4** | 1 | 0.25 | 1.25 | 1:125 |
| **5** | 1 | 0.25 | 1.25 | 1:625 |
| **6** | 1 | 0.25 | 1.25 | 1:3125 |

* **Results**

**Table 2.Absorbance for Nuclear fraction**

|  |  |  |
| --- | --- | --- |
| Tube Number | Absorbance | DNA concentration |
| N1 | 20.02 |  |
| N2 | 1.442 | 14.42 |
| N3 | 0.495 | 9.9 |
| N4 | 0.280 | 0.112 |
| N5 | 0.239 | 0.0192 |
| N6 | 0.236 | 0.00377 |

**Table 3.Absorbance for Homogenate fraction**

|  |  |  |
| --- | --- | --- |
| Tube number | Absorbance | DNA concentration |
| H1 | 20.02 |  |
| H2 | 3.565 | 35.65 |
| H3 | 1.100 | 22 |
| H4 | 0.429 | 0.1716 |
| H5 | 0.273 | 0.0218 |
| H6 | 0.232 | 0.00371 |

**Table 4. Absorbance for mitochondrial fraction**

|  |  |  |
| --- | --- | --- |
| Tube number | Absorbance | DNA concentration |
| M1 | 2.310 |  |
| M2 | 0.722 | 2.888 |
| M3 | 0.336 | 6.72 |
| M4 | 0.262 | 0.1048 |
| M5 | 0.253 | 0.0202 |
| M6 | 0.251 | 0.0040 |

Each of the three tables shows a decrease in absorption due to dilution. We also note that the highest absorption was in homogenate, followed by nuclear, and the lowest in mitochondria.

**DNA concentration claculation**

***DNA Concentration* DNA concentration (µg/ml)=A260\*50 µg/ml\*dilution factor**

* **Discussion**

in this exbiriment we wanted to detremain the concentration of DNA,by measure the absorbance at 260nm for each tube of the fractions collected in the last experiment( Homogenate, Nuclear, Mitochondrial) in the spectrophotometer.

Befor measure the absorbance we was did a seral dilution, The absorbance in the tubes decrease with increase the dilution ratio , and this is what we noticed through the absorbance readings in the tabels 2,3,and 4 .

The hithgist value for DNA concentration in Homoginate fraction becuse it contane all the organels , and the second concentration was in the Nucleare fraction,

And the lowest concentration of DNA was in Mitochondrial

Questions:

1-The early endosumbiosis between prokaryotic endosutosis and eukaryotic host cell gave rise to organelles such as mitochondria in eukaryotic cells.

2- Aceto orcein is a biological stain made consists of orcein in solution with acitic acid that is used to break apart the cellulose cell wall and crush tissue on a microscope slide. It converts chromosomal perpule to red hue.

3- rovide moredurability than plastic and it excels at transmiting UV light

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* **Refrances**

Pasquali, C., Fialka, I., & Huber, L. A. (1999). Subcellular fractionation, electromigration analysis and mapping of organelles. *Journal of Chromatography B: Biomedical Sciences and Applications*, *722*(1-2), 89-102.‏