

# Good essay on efficient isolation of pure and functional mitochondria from mouse ...

[Experience](#), [Failure](#)



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## **Introduction**

Mitochondrion plays vital roles in the performance of tissue. It is important, therefore, that their isolation and study are advanced to correct anomalies that arise out of the mitochondria failure. Instances of a mitochondria failure in neuronal tissue have been reported to cause anomalies such as epilepsy and ageing. Since mitochondria is responsible for the production of ATP in the cells, failure or inefficiency of these organelles causes a dysfunction in their component tissue leading to some ailment in the host organism. Many methods exist for the extraction and observation of mitochondria, some of these methods include, differential centrifugation and the recent Magnetic resonance spectroscopy.

The efficiency of the scientific method is determined by its ability to foster a good understanding of the question or aspect under research. Overall, experimental aspects such as procedures, analysis and rationale determine whether a method is credible or incredible.

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In one of the journal articles, authored by a number of scientists including Eva Bergschneider, Andras Franko and Olivier Baris, “ Efficient Isolation of Pure and Functional Mitochondria from Mouse Tissue Using Automated Tissue Disruption and Enrichment with Anti-TOM22 Magnetic Beads, ” the authors propose the use of Anti-TOM22 Magnetic Beads in investigating molecular mechanisms . This is in comparison to the long established methods of studying molecular mechanisms. Therefore, this paper seeks to analyze the validity and hence the credibility of this method in comparison to other methods used in the past.

## **Background**

Mitochondria play a big role in the functionality of the body. The major function is the production of ATP (energy). In addition to production of energy, there are other functions that the mitochondria play which are also of great significance to the body. These include cell deaths, cell differentiating, signaling and controlling the cell cycle and cell growth. The above functions play a big role in the aging process. Mitochondrion as an organelle has specific compartments that carry out various functions. The major functions played by the mitochondrion as an organelle is steroid synthesis, regulation of the cellular redox state, heme synthesis, cellular proliferation, apoptosis-programmed cell growth, regulation of the membrane potential, glutamate-mediated excitotoxic neuronal injury and the regulation of the membrane potential. Mitochondria acquire their own genetic material and the ability to manufacture their own RNAs and proteins. A human being has 13 mitochondrial peptides that are integrated into the

inner mitochondrial membrane alongside proteins set by genes that reside in the host cell's nucleus. Aerobic respiration takes place in the mitochondria completing the breakdown of glucose to release energy used by the cells. The mitochondrion has three compartments each carrying out its own function. These are the inner membrane, the cristae and the matrix. There is great scientific evidence that shows abnormalities within the mitochondria causes many human disorders and diseases. As such, modern scientific studies have developed many methods that seek to dig deeper into the understanding of how mitochondria works and subsequently find out how alterations within the mitochondrion could be the root to diseases.

## **Experimental rationale**

The hypothesis being tested in this paper was the comparative advantage of Anti-TOM22 Magnetic Beads over traditional methods such differential centrifugation protocol in the isolation of mitochondria from other cell organelles. The results were analyzed using the quality and quantity of the isolated mitochondria and subsequently comparisons between the two methods done. In their propositions, authors of this paper argued - that Anti TOM22 Magnetic Beads proves a more credible and reliable method due to the precision and accuracy which is occasioned by the application of hi-tech methods of isolation and studying molecular mechanisms . However, the hypothesis of the paper was very narrow and is only result-oriented. In comparison, the paper should have looked at other experimental aspects such as the cost and time.

## **Methodology used**

The use of ultra modern devices in this experiment played a big role in the accuracy of the results. The XF Extracellular Flux Analyzer used to measure oxygen utilization in several samples, including isolated mitochondria, is one of the most modern devices. It allows the examination of mitochondrial function in smaller samples and a decidedly parallel fashion. This device is far much better compared to the traditional devices such as the Clark electrode which measured oxygen consumption in small sample one by one. It could not measure the samples simultaneously. Application of the gradient centrifugation method which augers well with the modern devices in analyzing mitochondrial respiration was significant to this experiment since it is a standardized isolation method allowing an instantaneous handling of huge samples. However, this method proves to be a bit difficult and cannot be performed fast since it consumes a lot of time. Alternatively the experiment would have applied the differential centrifugation method which is much simpler and consumes less time compared to the gradient centrifugation method. Moreover, this method results to mitochondrial fractions presenting good purity unlike the gradient centrifugation method which yields mitochondrial fractions lacking synaptosomal contaminations. A method which is easy to use, reproducible, and appropriate for a variety of mouse tissues and most significantly avoids non-mitochondrial contaminations would be the most appropriate for this experiment. Alternatively, the researchers would have considered using guinea pigs or volunteer subjects in the experiment. These would result to similar results as that of the mice that are preferred in most biological experiments. The

researchers could also incorporate skeletal tissues in the experiment as an addition to the tissues normally used that is the brain, liver and heart tissues.

## **Results/interpretation**

Liver, brain and heart tissues were used to test the credibility of the new protocol. The first step was to check the mitochondria for the presence of contamination from other cell organelles. As compared to the other methods, the new protocol showed no contamination from other cell organelles such as the endoplasmic reticulum. Mitochondria isolated from mouse tissues were tested for intactness. As opposed to other methods that have been applied in isolation processes, this protocol showed a significant level of increased intactness of 90% that is quite credible. Purity tests were also done to identify the amount of Cytochrome c in liver cells of mice. This new protocol showed a high level of purity with very small amounts of Cytochrome c present. Anti TOM22 Magnetic Beads showed a high level of purity compared to differential centrifugation. However; there are some critical aspects, which the research and subsequently the results failed to analyze. The cost and time of each protocol were two fundamental aspects of comparison that the paper left out.

## **Conclusion**

The study of molecular mechanisms is a very vital discipline in modern medicine. This method has been able to pin down the origin of some complications and subsequently offered diagnostic hints to health complications. Anti TOM22 Magnetic Beads is a credible protocol for isolation

since it provides higher chances of precision, clarity and purity. However, more research needs to be done to incorporate principles behind all the protocols in order to develop an even robust method of isolating cellular bodies for the purpose of research.