

# Protocol for Using Microfluidics in Extracting Functionally Competent Mitochondria

## Introduction

Mitochondria, often referred to as the powerhouses of the cell, are critical organelles responsible for producing the energy necessary for cellular functions. The isolation of functionally competent mitochondria is pivotal for the study of bioenergetics and understanding the links between energy, health, and disease (PubMed, 2023). Recent advancements in microfluidic technology have provided innovative methods for the isolation of mitochondria, offering several advantages over traditional techniques such as differential centrifugation. This report outlines a detailed protocol for using microfluidics to extract functionally competent mitochondria, drawing on the latest research and methodologies in the field.

## Microfluidic Technology in Mitochondrial Isolation

Microfluidic devices have revolutionized various aspects of biological research due to their high-throughput capabilities, cost-effectiveness, and minimal sample requirements. These devices exploit fluid dynamics at the microscale to manipulate small volumes of fluids in channels with dimensions of tens to hundreds of micrometers. In the context of mitochondrial isolation, microfluidic platforms offer a gentle yet efficient alternative to traditional methods, which can be time-consuming and may compromise mitochondrial integrity (PubMed, 2023; Biomed Central, 2023).

## Advantages of Microfluidic Mitochondrial Isolation

The use of microfluidic devices for mitochondrial isolation presents several advantages:

1. **Reduced Physical Trauma:** Microfluidic devices cause less physical trauma to cells and organelles, preserving the physiological environment of the mitochondria (PubMed, 2023).
2. **High-Throughput:** Microfluidic techniques are capable of processing multiple samples simultaneously, increasing the throughput of mitochondrial isolation (PubMed, 2023).
3. **Cost-Effectiveness:** The devices themselves are inexpensive, and the microscale operation reduces the consumption of reagents (Biomed Central, 2023).
4. **Quality of Isolation:** Microfluidic platforms can yield mitochondria with intact membrane potential and functional electron transport chains, which are crucial for downstream applications (PubMed, 2023).

## Protocol for Microfluidic Mitochondrial Isolation

### Materials and Equipment

- Microfluidic device designed for mitochondrial isolation
- Cell culture flasks (T175)
- Homogenization buffer (300 mM sucrose, 10 mM K-HEPES [pH 7.2], 1 mM K-EGTA [pH 8.0])

- Syringe filters (0.22- $\mu$ m and 0.20- $\mu$ m)
- gentleMACS Dissociator (Miltenyi Biotec)
- Subtilisin A (Sigma-Aldrich, #P5380)
- Differential filtration system with optimized filters
- Flow cytometry equipment

## Sample Preparation

1. **Cell Culture:** Cultivate cells in T175 culture flasks until a sufficient number of cells are obtained (approximately  $2.5\text{--}3.5 \times 10^7$  cells/flask).
2. **Cell Collection:** Harvest and pellet the cells by centrifugation.

## Cell Lysis and Mitochondrial Isolation

1. **Resuspension:** Resuspend the cell pellet in 2 ml of homogenization buffer, filtered through a 0.22- $\mu$ m or 0.20- $\mu$ m syringe filter.
2. **Homogenization:** Transfer the suspension to gentleMACS C tubes and homogenize using the gentleMACS Dissociator's pre-programmed mitochondrial setting.
3. **Enzymatic Treatment:** Add 1 mg of Subtilisin A to each sample and mix by inversion to aid in the breakdown of extracellular matrix components.

## Microfluidic Processing

1. **Device Loading:** Introduce the cell lysate into the microfluidic device, which is designed to apply shear forces to release mitochondria from the cell debris.
2. **Mitochondrial Extraction:** Operate the device according to the manufacturer's instructions to isolate mitochondria based on size exclusion or other microfluidic principles.

## Mitochondrial Purification

1. **Filtration:** Use differential filtration with optimized filters to remove whole cell contaminants and further purify the mitochondrial fraction.
2. **Quality Control:** Employ flow cytometry to count live mitochondria and assess purity.

## Validation of Isolated Mitochondria

1. **Functional Assessment:** Measure the respiration rate of the isolated mitochondria under various conditions to ensure functional competence.
2. **Structural Integrity:** Confirm the structural integrity of the mitochondria using transmission electron microscopy (TEM) or similar techniques.

## Conclusion

The microfluidic approach to mitochondrial isolation offers a promising alternative to traditional methods, providing high-quality and functionally competent mitochondria suitable for a wide range of biomedical applications. By following the outlined protocol, researchers can efficiently isolate mitochondria while preserving their structural and functional integrity, which is essential for advancing our understanding of mitochondrial roles in health and disease.

## References

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