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Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components	1.25 mL Anti-TOM22 MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-TOM22 antibodies.
Capacity	For 25 separations each with 50–100 mg tissue or with up to 10^7 cells. ▲ Note: Please note that the referred capacity might vary for applications that require a different concentration of Anti-TOM22 MicroBeads.
Product format	Anti-TOM22 MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS® Technology for mitochondria isolation

Subcellular fractionation, e.g., isolation of mitochondria, is typically performed by density gradient centrifugation of cell and tissue homogenates. This technique is both time-consuming and labour-intensive. Alternatively, differential centrifugation is used as a much faster method, but resulting in a decreased mitochondria purity compared to density gradient centrifugation.

In contrast to density gradient centrifugation, MACS® Technology accelerates the isolation process and enables easy isolation of mitochondria from mouse tissue. Mitochondria isolation based on MACS Technology results in higher recoveries compared to common methods like density gradient centrifugation. This is of special particular advantage when working with limiting amounts of starting material.¹

Using the Mitochondria Isolation Kit, mouse tissue (# 130-096-946) cells are lysed and mitochondria are magnetically labeled with Anti-TOM22 MicroBeads, mouse. The monoclonal Anti-TOM22 antibody specifically binds to the translocase of outer mitochondrial membrane 22 (TOM22) of mouse mitochondria. Next, the labeled tissue lysate is passed through a 30 µm filter and loaded onto a MACS Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled mitochondria are retained within the column. The unlabeled organelles and cell components run through. After removing the column from the magnetic field, the magnetically retained mitochondria can be eluted (refer to figure 1).

1.2 Background information

Mitochondria are organelles, which range in size between 0.5–2 micrometers in length. They occur in numbers that directly correlate with the cell's level of metabolic activity. Mitochondria can be considered the power generators of the cell, converting oxygen and nutrients into adenosine triphosphate (ATP) and therefore play a crucial role in cellular energy production and metabolism.

Mitochondria are supposed to play a central role in aging-related neurodegenerative diseases^{2–4}, in Diabetes Mellitus^{5,6} as well as heart failure⁷ or cancer^{8,9}. Mutations in mitochondrial genes can lead to a number of mitochondrial disorders and the muscle or brain are most commonly affected since they rely heavily on mitochondria for their energy needs.¹⁰

For proper analysis of mitochondria an easy and reliable procedure for mitochondria isolation is important. Anti-TOM22 MicroBeads, mouse have been developed for the separation of mitochondria from mouse tissue to facilitate mitochondria research when working with animal models of disease.

1.3 Applications

- Anti-TOM22 MicroBeads, mouse are to be used in combination with the Mitochondria Isolation Kit, mouse tissue (# 130-096-946) for applications that require a higher concentration of Anti-TOM22 MicroBeads than those provided in the kit.

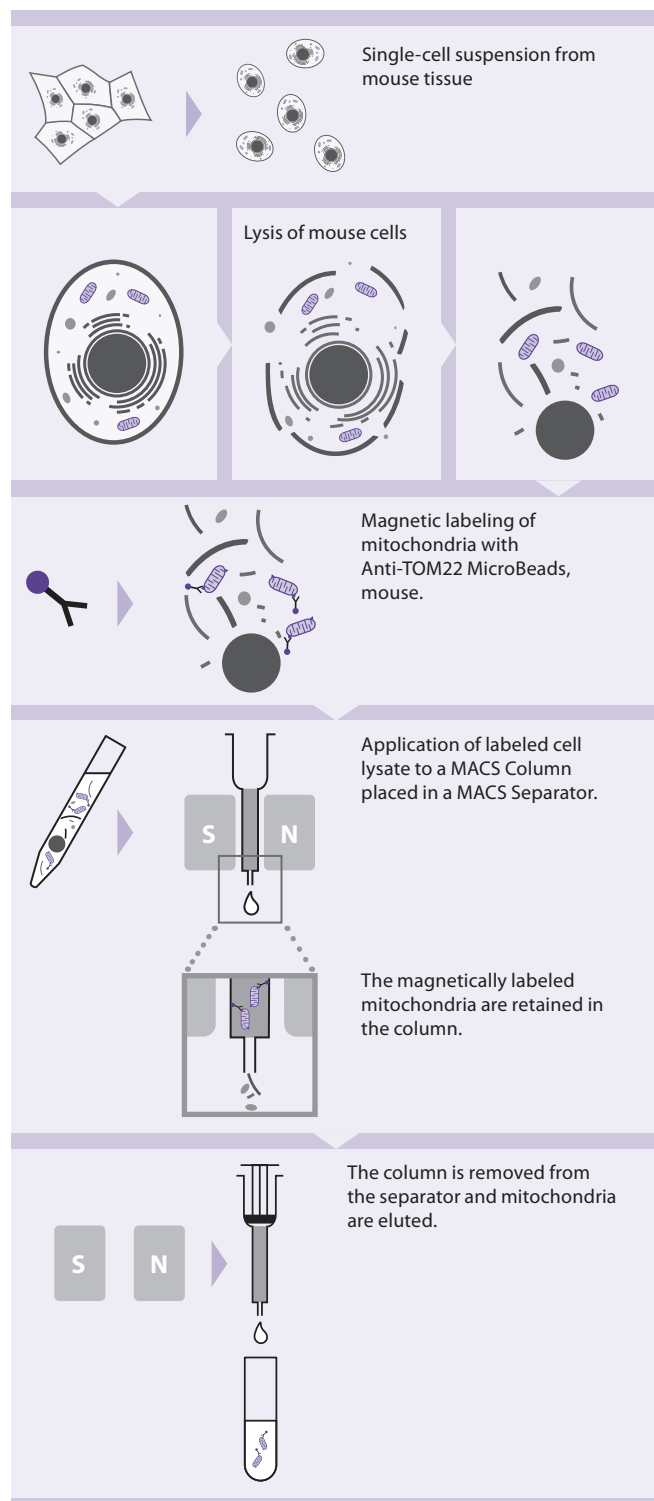


Figure 1: Isolation of mitochondria from mouse tissue using MACS Technology.

1.4 Reagent and instrument requirements

Before preparation of tissue lysate

- (Optional) Mitochondria Extraction Kit – Tissue (# 130-097-340) to homogenize tissue for mitochondrial extraction

Preparation of tissue lysate

- Mitochondria Isolation Kit, mouse tissue (# 130-096-946)
- Cooled centrifuge
- Protease inhibitors, reconstitute in phosphate buffered saline (PBS), pH 7.2
- gentleMACS™ Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937), alternatively a dounce homogenizer
- Ice bucket

MACS® Separation using Anti-TOM22 MicroBeads, mouse

- Mitochondria Isolation Kit, mouse tissue (# 130-096-946)
- MidiMACS™ Separation Unit (# 130-042-302) or QuadroMACS™ Separation Unit (# 130-090-976)
- MACS® MultiStand (# 130-042-303)
- Ultrapure water
- Orbital shaker / end-over-end shaker or the MACSmix™ Tube Rotator (# 130-090-753)
- (Optional) Pre-Separation Filters (70 µm) (# 130-095-823) to remove large insoluble tissue fragments.
- (Optional) Cooled table-top centrifuge

2. Protocol

▲ For the mitochondria isolation protocol, please refer to the protocol specified in the data sheet of the Mitochondria Isolation Kit, mouse tissue (# 130-096-946).

▲ For the isolation of mitochondria from mouse brain, please refer to the application note “**Synaptic mitochondria isolation**” and to the publication from Hubbard *et al.*¹¹

3. References

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Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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