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1. Description

Components	<p>1.25 mL Anti-TOM22 MicroBeads, human: MicroBeads conjugated to monoclonal anti-TOM22 antibodies (isotype: mouse IgG1).</p> <p>25 mL Lysis Buffer</p> <p>56.25 mL 10× Separation Buffer</p> <p>27.50 mL Storage Buffer</p> <p>25 LS Columns</p>
Capacity	For 25 separations, each with up to 10 ⁷ cells or appropriate amount of tissue.
Product format	Anti-TOM22 MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	<p>Store Anti-TOM22 MicroBeads, Lysis Buffer, and Storage Buffer protected from light at 2–8 °C. Do not freeze.</p> <p>Bring 10× Separation Buffer to room temperature and aliquot directly after arrival. Prepare 2.3 mL aliquots (needed volume per reaction is 2.25 mL) and store at –20 °C.</p> <p>Store LS Columns dry and protected from light at room temperature.</p> <p>The expiration dates are indicated on the label.</p>

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 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 human cells or 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, cells are lysed and mitochondria are magnetically labeled with Anti-TOM22 MicroBeads, first. The monoclonal Anti-TOM22 antibody specifically binds to the translocase of outer mitochondrial membrane 22 (TOM22) of human mitochondria. Then, the labeled cell lysate is 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 generally oblong 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, in Diabetes Mellitus as well as heart failure or cancer. 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.

For proper analysis of mitochondria an easy and reliable procedure for mitochondria isolation is important. Anti-TOM22 MicroBeads have been developed for the separation of mitochondria from human cells or tissue to facilitate mitochondria research.

1.3 Application

- Isolation of intact, vital mitochondria from human cells or tissue.

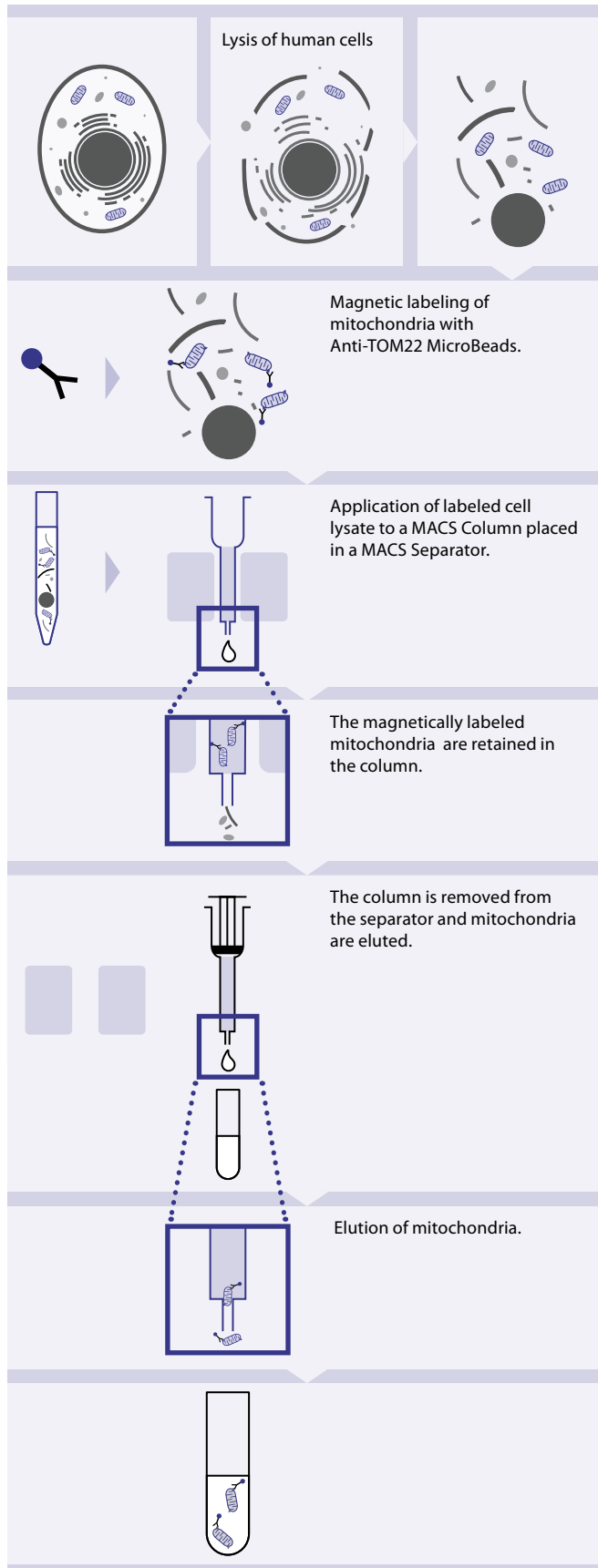


Figure 1: Principle of the MACS Technology for the isolation of human mitochondria

1.4 Reagent and instrument requirements

Preparation of cell lysate

- Phosphate buffered saline (PBS), pH 7.2
- Cooled centrifuge
- Protease inhibitors, reconstitute in PBS, pH 7.2
- Dounce homogenizer
or
 - 26 G needle (e.g. Sterican® 0.45×25 mm, 26 G×1", Gr.18, B. Braun # 46507683) and 1 mL syringe (e.g. 1 mL HSW NORM-JECT®, Henke-Sass, Wolf GmbH # 4010.200V0)
 - or
 - 29G needle and syringe (e.g. BD Micro-Fine™, U-100 insulin, 0.33 mm (29G)×12.7 mm, BD # 320411)
- Ice bucket

MACS® Separation using Anti-TOM22 MicroBeads

- 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 (# 130-041-407) to remove cell clumps.
- (Optional) Cooled table-top centrifuge.

2. Protocol

2.1 Preparation of cell lysate

▲ Perform the lysis on ice to reduce the activity of damaging proteases and phospholipases. Pre-cool PBS and Lysis Buffer on ice to 4 °C and prepare an ice bucket.

▲ Protease inhibitors should be added to the lysis buffer.

1. Harvest your cells of interest.
2. Centrifuge cell suspension at 300×g for 10 minutes at 4 °C. Aspirate the supernatant.
3. Resuspend cell pellet in appropriate amount of ice-cold PBS (10 mL/10⁷ cells).
4. Determine cell number by using a hemocytometer.
5. Centrifuge cell suspension at 300×g for 10 minutes at 4 °C. Aspirate the supernatant.
6. Resuspend cell suspension in 1 mL ice-cold Lysis Buffer per 10⁷ total cells.
7. Homogenize the cells by using a dounce homogenizer or a needle.

▲ It is highly important to determine the optimal stroke number for each cell line, due to their different sizes and constitutions.

▲ **Note:** Perform cell homogenization with a dounce homogenizer or a needle stepwise using 5,10, 15, and 20 strokes. Analyze the amount of lysed cells using a hemocytometer and determine optimal stroke number.

▲ **Note:** Especially if mitochondria are used in functional assays we strongly advise to examine the integrity of these organelles after the lysis procedure, e.g., by measuring citrate synthase activity.

- Directly proceed to magnetic labeling (2.2).



2.2 Magnetic labeling

▲ Dilute 10× Separation Buffer with bidistilled water (H₂O bidest) to achieve 1× Separation Buffer. Pre-cool 1× Separation Buffer on ice to 4 °C. Keep cell lysate on ice.

- Transfer 1 mL of lysate (10⁷ cells/mL) to a 15 mL conical tube.
- Add 9 mL of ice-cold 1× Separation Buffer. Mix well.
- Add 50 µL Anti-TOM22 MicroBeads to magnetically label the mitochondria.
- Mix well and incubate for 1 hour in the refrigerator (2–8°) with gentle shaking, e.g., using the MACSmix Tube Rotator.
- Directly proceed to magnetic separation (2.3).



2.3 Magnetic separation

▲ Always wait until the column reservoir is empty before proceeding to the next step.

- Place one LS Column per separation in the magnetic field of a suitable MACS Separator (MidiMACS Separation Unit or QuadroMACS Separation Unit).
- Prepare column by rinsing with 3 mL of 1× Separation Buffer.
- After labeling incubation (refer to 2.2, step 5) has finished apply the cell lysate onto the column stepwise (3×3.3 mL) and let the lysate run through.

▲ **(Optional):** If cell clumps or aggregates are visible use a Pre Separation Filter (# 130-041-407) to remove them from the cell lysate.

- Wash column with 3×3 mL of 1× Separation Buffer.

▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
- Remove column from the separator and place it on a suitable collection tube (15 mL conical tube).
- Pipette 1.5 mL of 1× Separation Buffer onto the column. Immediately flush out the magnetically labeled mitochondria by firmly pushing the plunger into the column. Immediately proceed with downstream analysis.

▲ **Note:** The 1× Separation Buffer contains stabilizing agent. Before measuring of mitochondria protein content please carry out the following procedure:

- Centrifuge the isolated mitochondria at 13000×g for 2 minutes at 4 °C. Aspirate the supernatant.
- Resuspend the mitochondria pellet in 1000 µL of Storage Buffer.
- Centrifuge mitochondria suspension at 13000×g for 2 minutes at 4 °C. Aspirate the supernatant.
- Resuspend the mitochondria pellet in 100 µL Storage Buffer.

- (Optional) If mitochondria are not immediately used for downstream analysis centrifuge mitochondria suspension at 13000×g for 2 minutes at 4 °C. Aspirate the supernatant. Resuspend the mitochondria pellet in 100 µL Storage Buffer and store on ice.

3. Reference

- Hornig-Do, H.T. *et al.* (2009) Isolation of functional pure mitochondria by superparamagnetic microbeads. *Anal. Biochem.* 389: 1–5.

All protocols and data sheets are available at www.miltenyibiotec.com.

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.

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