Miltenyi Biotec

Mitochondria Isolation Kit
human

Order no. 130-094-532

Contents

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, human:** MicroBeads conjugated to monoclonal anti-TOM22 antibodies. **25 mL Lysis Buffer** **56.25 mL 10× Separation Buffer** **27.50 mL Storage Buffer** **25 LS Columns**
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	Store Anti-TOM22 MicroBeads, Lysis Buffer, and Storage Buffer protected from light at 2–8 °C. Do not freeze. Incubate 10× Separation Buffer to 37 °C until the crystals are no longer visible and aliquot directly upon arrival. Prepare 2.3 mL aliquots (needed volume per reaction is 2.25 mL) and store at –20 °C. Store LS Columns dry and protected from light at room temperature. The expiration date is indicated on the 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 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 Applications

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

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-002-697.07

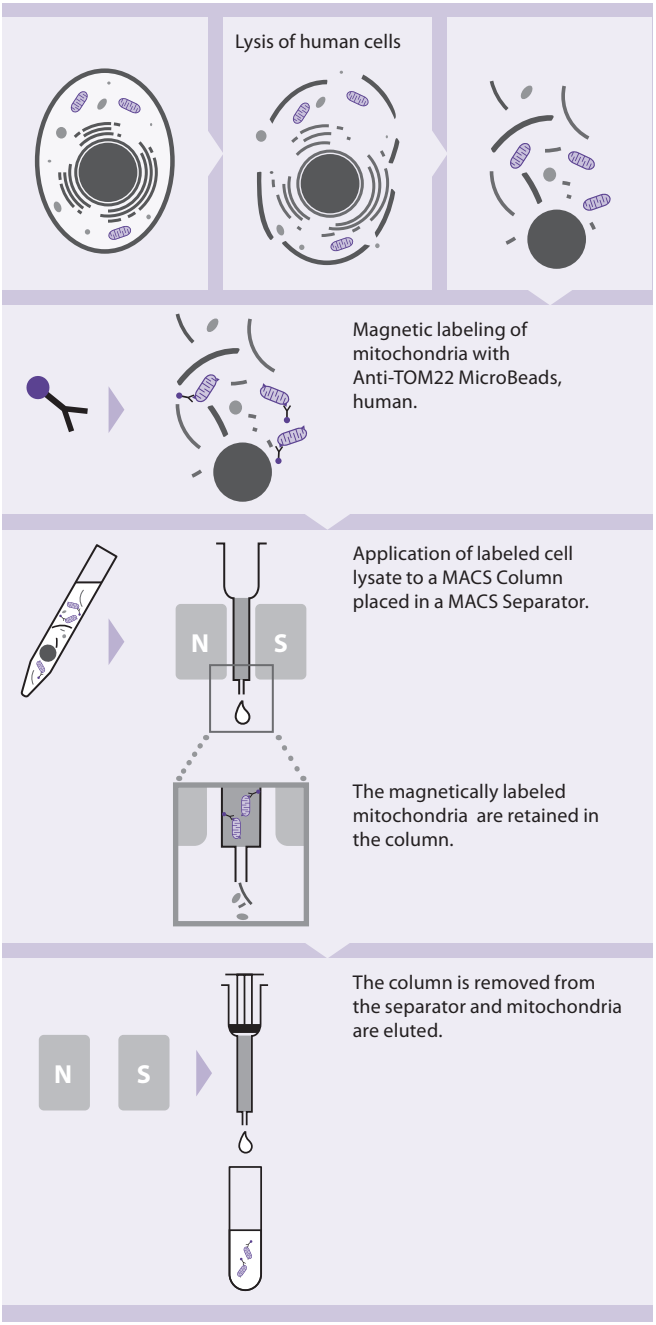

Figure 1: Isolation of human mitochondria using MACS Technology.

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) for large cells, e.g., HEK 293 cells

  or
  - 29 G needle and syringe (e.g. BD Micro-Fine™, U-100 insulin, 0.33 mm (29G) × 12.7 mm, BD # 320411) for small cells, e.g. Jurkat cells
- 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 (30 µm) (# 130-041-407) to remove cell clumps.
- (Optional) Cooled table-top centrifuge

2. Protocol

2.1 Sample preparation

▲ 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 appropiate amount of ice-cold PBS (10 mL/10^7 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 10^7 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 that the integrity of these organelles be examined after the lysis procedure, especially if the mitochondria are to be used in functional assays, e.g., by measuring citrate synthase activity.
8. Directly proceed to magnetic labeling (2.2).

140-002-697.07

2.2 Magnetic labeling

▲ Dilute 10× Separation Buffer with bidistilled water (H_2O bidest) to achieve 1× Separation Buffer. Pre-cool 1× Separation Buffer on ice to 4 °C. Keep cell lysate on ice.

1. Transfer 1 mL of lysate (10^7 cells/mL) to a 15 mL conical tube.
2. Add 9 mL of ice-cold 1× Separation Buffer. Mix well.
3. Add 50 µL Anti-TOM22 MicroBeads to magnetically label the mitochondria.
4. Mix well and incubate for 1 hour in the refrigerator (2–8°) with gentle shaking, e.g., using the MACSmix Tube Rotator.
5. 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.

1. Place one LS Column per separation in the magnetic field of a suitable MACS Separator (MidiMACS Separation Unit or QuadroMACS Separation Unit).
2. Prepare column by rinsing with 3 mL of 1× Separation Buffer.
3. After labeling incubation (refer to 2.2, step 4) has finished apply the cell lysate onto the column stepwise (3×3.3 mL) and let the lysate run through.
4. (Optional) If cell clumps or aggregates are visible use a Pre-Separation Filter (30 µm, # 130-041-407) to remove them from the cell lysate.
5. Wash column with 3×3 mL of 1× Separation Buffer.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
6. Remove column from the separator and place it on a suitable collection tube (15 mL conical tube).
7. 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:

   1. Centrifuge the isolated mitochondria at 13,000×g for 2 minutes at 4 °C. Aspirate the supernatant.
   2. Resuspend the mitochondria pellet in 1,000 µL of Storage Buffer.
   3. Centrifuge mitochondria suspension at 13,000×g for 2 minutes at 4 °C. Aspirate the supernatant.
   4. Resuspend the mitochondria pellet in 100 µL Storage Buffer.
8. (Optional) If mitochondria are not immediately used for downstream analysis centrifuge mitochondria suspension at 13,000×g for 2 minutes at 4 °C. Aspirate the supernatant. Resuspend the mitochondria pellet in 100 µL Storage Buffer and store on ice.

3. References

1. Hornig-Do, H.T. *et al.* (2009) Isolation of functional pure mitochondria by superparamagnetic microbeads. Anal. Biochem. 389: 1–5.
2. Minet, A.D. and Gaster, M. (2011) The dynamic equilibrium between ATP synthesis and ATP consumption is lower in isolated mitochondria from myotubes established from type 2 diabetic subjects compared to lean control. Biochem. Biophys. Res. Comm. 409: 591–595.
3. Minet, A.D. and Gaster, M. (2010) ATP synthesis is impaired in isolated mitochondria from myotubes established from type 2 diabetic subjects. Biochem. Biophys. Res. Comm. 402: 70–74.
4. Guo, T. *et al.* (2010) Quantitative proteomics discloses MET expression in mitochondria as a direct target of MET kinase inhibitor in cancer cells. Mol. Cell. Proteomics 9: 2629–2641.
5. Eriksen, M.B. *et al.* (2011) Intact primary mitochondrial function in myotubes established from women with PCOS. J. Clin. Endocrinol. Metab. 96: E1298–1302.
6. Barrey, E. *et al.* (2011) Pre-microRNA and mature microRNA in human mitochondria. PLoS ONE 6: e20220.
7. Bandiera, S. *et al.* (2011) Nuclear outsourcing of RNA interference components to human mitochondria. PLoS ONE 6: e20746.
8. Sacchi, S. *et al.* (2011) Evidence for the interaction of d-amino acid oxidase with pLG72 in a glial cell line. Mol. Cell. Neurosc. 48: 20–28.

4. Related products

Mitochondria MidiMACS Starting Kit, human	# 130-094-872
Mitochondria QuadroMACS Starting Kit, human	# 130-094-833
Mitochondria Isolation Kit, mouse tissue	# 130-096-946
Mitochondria MidiMACS Starting Kit, mouse tissue	# 130-097-039
Mitochondria QuadroMACS Starting Kit, mouse tissue	# 130-097-040
gentleMACS™ Dissociator	# 130-093-235
gentleMACS Octo Dissociator	# 130-095-937
Neural Tissue Dissociation Kit (P)	# 130-092-628
Neural Tissue Dissociation Kit (T)	# 130-093-231
Tumor Dissociation Kit, human	# 130-095-929
Tumor Dissociation Kit, mouse	# 130-096-730
Brain Tumor Dissociation Kit (P), human	# 130-095-942

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.

Legal notices

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.
All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.
The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.
CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

gentleMACS, MACS, MACSmix, MidiMACS, the Miltenyi Biotec logo, and QuadroMACS are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

BD Micro-Fine is a trademark of Becton, Dickinson and Company.
HSW NORM-JECT is a registered trademark of Henke-Sass, Wolf GmbH.
Sterican is a registered trademark of B. Braun Melsungen AG.