Miltenyi Biotec

Mitochondria Isolation Kit

mouse tissue

Order no. 130-096-946

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, mouse:** 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** **25 Pre-Separation Filters (30 µm)**
Capacity	For 25 separations each with 50–100 mg tissue or with up to 10^7 cells.
Product format	Anti-TOM22 MicroBeads, mouse are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store Anti-TOM22 MicroBeads, mouse, 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 and Pre-Separation Filters (30 µm) 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 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, 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 failure[7] 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.

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

140-003-459.03

1.3 Application

- Isolation of intact, vital mitochondria from mouse tissue.

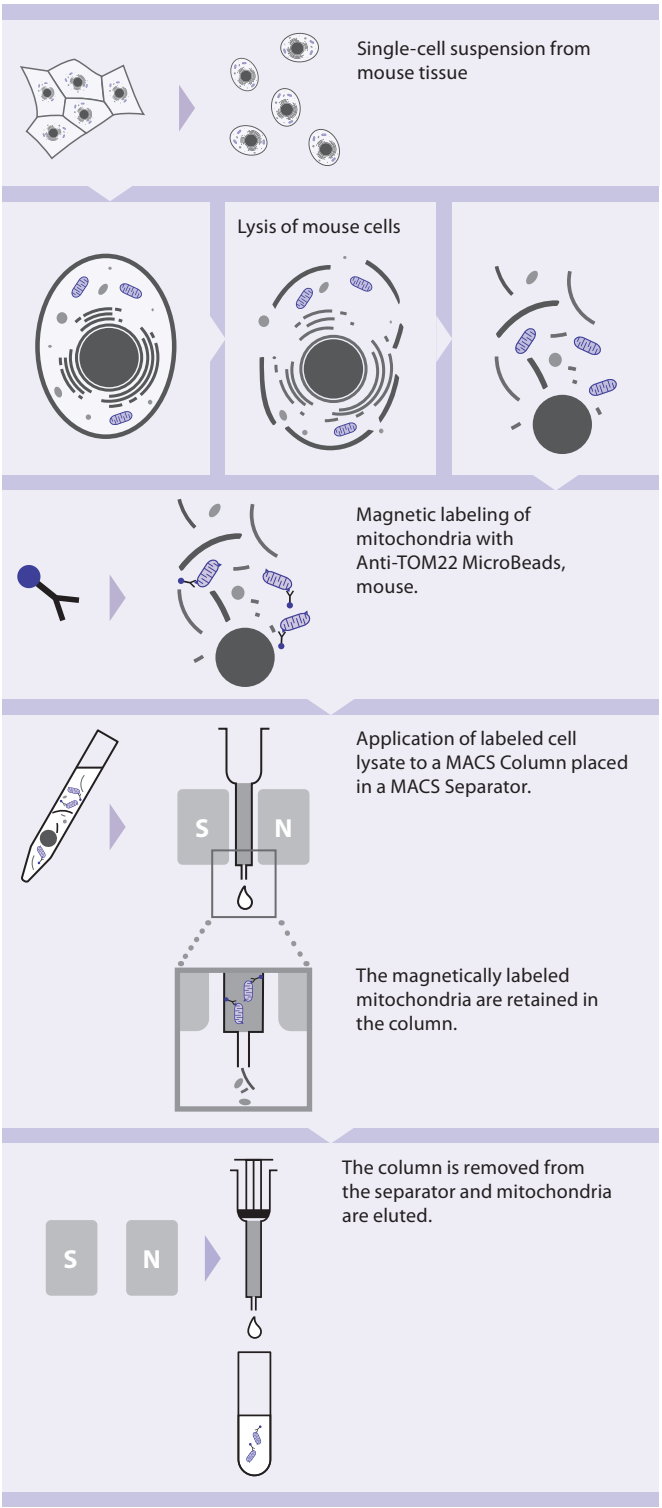

Figure 1: Isolation of mitochondria from mouse tissue using MACS Technology.

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

- Lysis Buffer
- 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

- 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

2.1 Preparation of tissue lysate

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

▲ If an enzymatic dissociation of the tissue is performed, wash the tissue with ice-cold Lysis Buffer and add protease inhibitors before cell lysis.

1. Homogenize the pre-weighed tissue by using the gentleMACS Dissociator using an appropriate mouse tissue mitochondria dissociation protocol. For more information refer to www.gentlemacs.com/protocols. Alternatively use a dounce homogenizer.
2. (Optional) Place a Pre-Separation Filter (70 µm) on a 15 mL conical tube placed on ice. Pipette the tissue homogenate into the reservoir of the filter and allow the homogenate to run through. Thoroughly collect the homogenate and store it on ice.

   ▲ **Note:** It is strongly recommend that the integrity of the mitochondria is examined after the lysis procedure, especially if the mitochondria are to be used in functional assays, e.g., by measuring citrate synthase activity.
3. Directly proceed to magnetic labeling (2.2).

140-003-459.04

2.2 Magnetic labeling

▲ Dilute 10× Separation Buffer with ultrapure 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 1–2 mL of lysate (derived from 50–100 mg dry tissue weight) to a 15 mL conical tube.
2. Add ice-cold 1× Separation Buffer to a final volume of 10 mL. Mix well.

   ▲ **Note:** If a buffer containing polysaccharides is used to lyse tissues, this must be diluted by ≥5-fold (maximum 2 mL in 10 mL) otherwise separation performance will be impaired.
3. Add 50 µL Anti-TOM22 MicroBeads, mouse to magnetically label the mitochondria.
4. Mix well and incubate for 1 hour in the refrigerator (2–8°C) 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.

Magnetic separation with LS Columns

1. Place column 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. Place a Pre-Separation Filter (30 µm) on top of the LS Column
4. After labeling incubation (refer to 2.2, step 4) has finished apply the labeled tissue homogenate into the reservoir of the filter onto the column stepwise (3×3.3 mL) and let the lysate run through.
5. Wash filter and column with 3×3 mL of 1× Separation Buffer.

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when 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 measurement 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. Wallace, D.C. (1999) Mitochondrial disease in man and mouse. Science. 283: 1482–1488.
3. Sergeant, N. *et al.* (2003) Association of ATP synthase α-chain with neurofibrillary degeneration in Alzheimer's disease. Neuroscience. 117: 293–303.
4. Van Remmen, H. and Richardson A. (2001) Oxidative damage to mitochondria and aging. Exp. Geront. 36: 957–968.
5. Kelley, D.E. *et al.* (2002) Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes. 51: 2944–2950.
6. Mogensen, M. *et al.* (2007) Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes. 56: 1592–1599.
7. Sharov, V.G. *et al.* (2000) Abnormal mitochondrial respiration in failed human myocardium. J. Mol. Cell. Cardiol. 32: 2361–2367.
8. Brandon, M. *et al.* (2006) Mitochondrial mutations in cancer. Oncogene. 25: 4647–4662.
9. King, A. *et al.* (2006) Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. Oncogene 25: 4675–4682.
10. Filosto, M. *et al.* (2011) The role of mitochondria in neurodegenerative diseases. J. Neurol. DOI: 10.1007.

4. Related products

Mitochondria MidiMACS Starting Kit, mouse tissue	# 130-097-039
Mitochondria QuadroMACS Starting Kit, mouse tissue	# 130-097-040
Mitochondria Isolation Kit, human	# 130-094-532
Mitochondria MidiMACS Starting Kit, human	# 130-094-872
Mitochondria QuadroMACS Starting Kit, human	# 130-094-833
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
Brain Tumor Dissociation Kit (T), human	# 130-095-939

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.

140-003-459.04

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

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.