Miltenyi Biotec

Mitochondria Extraction Kit – Tissue

Order no. 130-097-340

Contents

1. Description

Components	**1 mL Solution 1** **50 mL 10× Solution 2** **52.5 mL Solution 3**
Capacity	For 25 extractions. The specified number of extractions is valid when processing tissue in the range of 50–100 mg following the protocol in chapter 2.2.2.
Storage	Store solutions protected from light at 2–8 °C. The expiration date is indicated on the labels.

1.1 Background information

Mitochondria dysfunction is involved in neurodegenerative diseases, such as Alzheimer or Parkinson, but also plays a pivotal role in diabetes, cancer, heart, kidney, or liver diseases. The Mitochondria Extraction Kit – Tissue has been developed for use with the gentleMACS™ Dissociators, allowing the time-saving and reliable homogenization of human or mouse tissues, including muscle, heart, liver, brain, or kidney. The resulting tissue homogenates provide an ideal basis for subsequent magnetic labeling and isolation of intact, functional mitochondria at high yield, using the Mitochondria Isolation Kit, human (# 130-094-532) or the Mitochondria Isolation Kit, mouse tissue (# 130-096-946).

1.2 Application

- Homogenization of human or mouse tissue for subsequent isolation of intact, functional mitochondria.

1.3 Reagent and instrument requirements

Tissue homogenization for mitochondrial extraction

- Human or mouse tissue
- Protease inhibitors (e.g. cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail Tablets provided in EASYpacks by Roche # 04 693 159 001)
- Cell culture dishes (35 mm)
- Dissection scissors and forceps
- gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- Pre-Separation Filters (70 µm) (# 130-095-823)

Additional reagents required for subsequent mitochondria isolation

- Mitochondria Isolation Kit, mouse tissue (# 130-096-946), containing: Anti-TOM22 MicroBeads, mouse, Lysis Buffer, 10× Separation Buffer, Storage Buffer, LS Columns, and Pre-Separation Filters (30 µm)

  or

  Mitochondria Isolation Kit, human (# 130-094-532), containing: Anti-TOM22 MicroBeads, human, Lysis Buffer, 10× Separation Buffer, Storage Buffer, and LS Columns
- For isolation of mitochondria from human tissue: Pre-Separation Filters, 30 µm (# 130-041-407)
- Cooled table-top centrifuge
- MACSmix™ Tube Rotator (# 130-090-753)
- MACS® MultiStand (# 130-042-303)
- MidiMACS™ Separator (# 130-042-302) or QuadroMACS™ Separator (# 130-090-976)
- 15 mL propylene conical tubes
- 1.5 mL microcentrifuge tubes

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2. Protocol for the preparation of mitochondria from tissue

2.1 Protocol overview

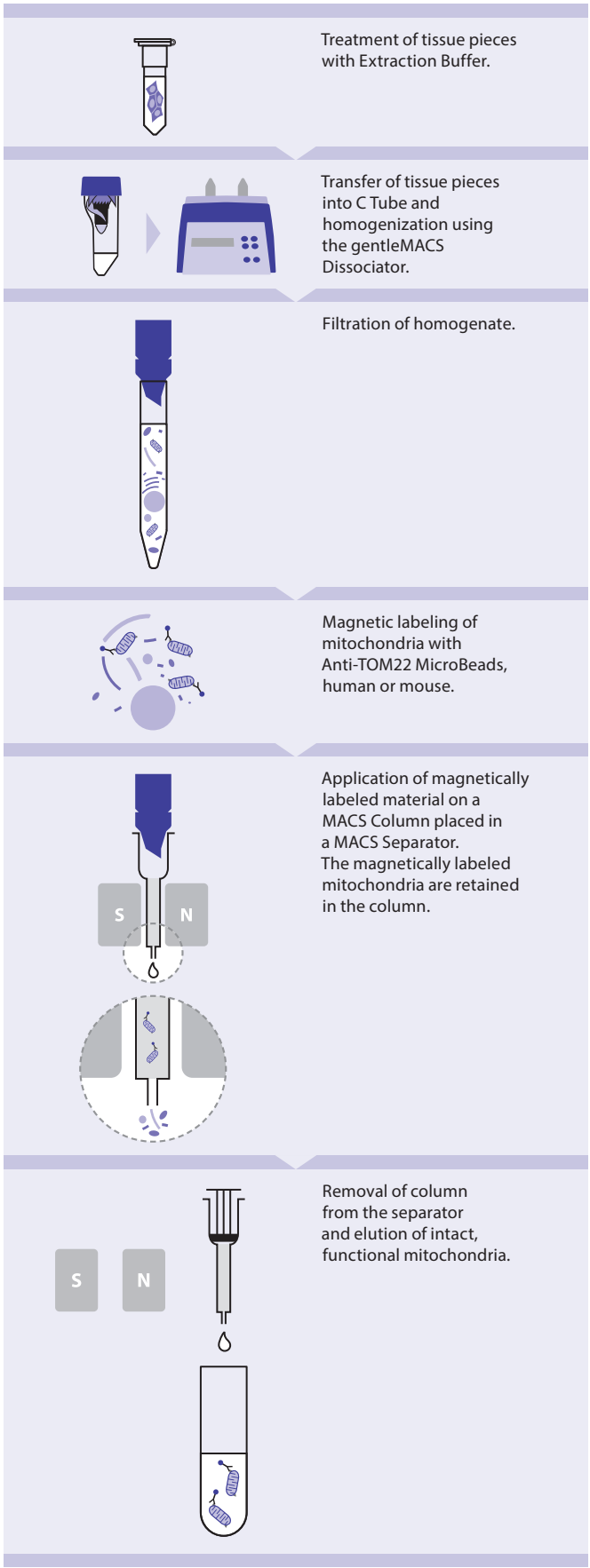

Figure 1: Isolation of mitochondria from human and mouse tissue.

2.2 Tissue homogenization for mitochondria extraction

▲ All buffers should be pre-cooled on ice before use.

2.2.1 Reagent preparation

1. Prepare 2.1 mL aliquots of Solution 3 and store at –20 °C.

   ▲ **Note:** 2 mL of Solution 3 are required for the homogenization of 50-100 mg tissue. Before use, thaw aliquot and keep on ice.

2. Prepare 10 mL Protease Inhibition Buffer: Add 1 mL of 10× Solution 2 to 9 mL double-distilled water and dissolve one protease inhibitor tablet in the freshly prepared 1× Solution 2.

   ▲ **Note:** 2 mL of Protease Inhibition Buffer are required for the homogenization of 50–100 mg tissue. Aliquots of 2 mL can be stored at -20 °C for 4 weeks.

3. Prepare 1× Solution 2 by adding 1 volume of 10× Solution 2 to 9 volumes of double-distilled water (H_20 bidest.), i.e., add 1.8 mL of 10× solution 2 to 16.2 mL of H_20 bidest.

   ▲ **Note:** Up to 18 mL of 1× Solution 2 are required for the homogenization of 50–100 mg tissue. Always use freshly prepared 1× Solution 2.

4. Prepare Extraction Buffer by adding 20 µL of Solution 1 to 0.5 mL of freshly prepared 1× Solution 2.

2.2.2 Tissue processing

▲ For details on the use of the gentleMACS Dissociators, refer to the gentleMACS Dissociator user manuals.

▲ Tissue in the range of 50–100 mg is homogenized in a C Tube in a total volume of 2 mL Protease Inhibition Buffer.

▲ **Note:** Up to 500 mg tissue in a maximum total volume of 10 mL can be dissociated per gentleMACS C Tube.

1. Rinse tissue twice in 3–4 mL ice-cold 1× Solution 2.
2. Add 3 mL ice-cold 1× Solution 2 to a 35 mm cell culture dish and place the dish on ice. Transfer tissue into the cell culture dish.

   ▲ **Note:** The tissue should be covered with liquid.

3. Resect fat, ligament, and connective tissue using a forceps and a pair of dissection scissors. Cut the tissue into pieces of approximately 5 mm.
4. Determine the weight of the tissue: Fill a second 35 mm cell culture dish with 3 mL ice-cold 1× Solution 2 and use this dish as tare weight. Quickly and gently dab the tissue pieces on a paper towel before placing them in the prepared culture dish. Measure the weight of the tissue.
5. Transfer 50–100 mg tissue to a 1.5 mL microcentrifuge tube containing 0.5 mL ice-cold Extraction Buffer.
6. Quickly mince tissue into small pieces of approximately 1–2 mm using a pair of dissection scissors. Incubate for 30 minutes on ice.
7. Centrifuge at 300×g for 5 minutes at 4 °C and discard the supernatant.
8. Add 1 mL ice-cold Protease Inhibition Buffer, gently flick the tube to resuspend the pellet and pour suspension into a pre-cooled gentleMACS C Tube. Rinse the microcentrifuge tube with an additional 1 mL of ice-cold Protease Inhibition Buffer and pour the suspension into the same gentleMACS C Tube.

   ▲ **Note:** Instead of pouring the suspension, a glass Pasteur pipette can also be used. Use of a plastic pipette tip is not recommended as small tissue pieces tend to stick to the wall of the tip.

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9. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.

   ▲ **Note:** It has to be ensured that the sample material is located in the area of the rotator/stator.

10. Run the gentleMACS Program.

    For mouse tissue: m_mito_tissue_01.

    For human tissue: h_mito_tissue_01.

11. After termination of the program, detach C Tube from the gentleMACS Dissociator and perform a short centrifugation step at 200×g for 30 seconds at 4 °C to collect the homogenate at the tube bottom.

12. Put a 15 mL conical tube on ice and place a Pre-Separation Filter (70 µm) on top. Remove homogenate from C Tube, pipette it into the reservoir of the filter and allow the homogenate to run through.

13. Wash filter with 2×1 mL ice-cold Solution 3. Centrifuge the filtered homogenate at 500×g for 5 minutes at 4 °C. Transfer supernatant containing mitochondria to a 15 mL conical tube and immediately proceed to 2.3 Mitochondria isolation.

2.3 Mitochondria isolation

▲ Magnetic labeling and separation is performed with homogenates derived from 50–100 mg tissue. Split homogenates derived from larger amounts of tissue into portions.

2.3.1 Reagent preparation

To prepare 23 mL of 1× Separation Buffer, incubate 10× Separation Buffer at 37 °C until visible crystals are no longer present and add 2.3 mL 10× Separation Buffer to 20.7 mL H_2O bidest. Pre-cool 1× Separation Buffer on ice.

▲ **Note:** Aliquots of 2.3 mL 10× Separation Buffer can stored at –20 °C.

2.3.2 Magnetic labeling and separation

1. Take supernatant derived from 50–100 mg tissue (section 2.1.2, step 13) and add ice-cold 1× Separation Buffer to a total volume of 10 mL. Mix well.

2. Add 50 µL Anti-TOM22 MicroBeads, mouse or 50 µL Anti-TOM22 MicroBeads, human to magnetically label the mitochondria.

3. Mix well and incubate for 1 hour in the refrigerator (2–8 °C) under continuous rotation using the MACSmix Tube Rotator.

   ▲ **Note:** Operate MACSmix Tube Rotator on permanent run at a speed of approximately 12 rpm.

4. Place LS Column into the magnetic field of a suitable MACS Separator and place a Pre-Separation Filter, 30 µm on top of the LS Column.

5. Prepare column by rinsing with 3 mL of 1× Separation Buffer.

6. Apply the magnetically labeled mitochondria onto the column and let the homogenate run through.

   ▲ **Note:** Apply homogenate in aliquots of 3×3.3 mL. Add aliquots only when the column reservoir is empty.

7. 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.

8. Remove column from the separator and place it on a 15 mL conical tube.

9. Pipette 1.5 mL of 1× Separation Buffer onto the column. Immediately flush out the enriched mitochondria by firmly pushing the plunger into the column.

10. 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.

11. (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 supplied with the Mitochondria Isolation Kit and store on ice. Storage should not exceed two hours to preserve high quality mitochondria. For functional analysis of mitochondrial respiration, optimal results are achieved when mitochondria are used immediately.

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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