Miltenyi Biotec

Skeletal Muscle Dissociation Kit
mouse and rat

Order no. 130-098-305

Contents

1. Description

This product is for research use only.

Components	7 vials, containing: **2 vials of Enzyme D (lyophilized powder)** **2.5 mL of Enzyme P** **2 vials of Enzyme A (lyophilized powder)** **2× 1 mL of Buffer A**
Size	For 50 digestions of 2.5 mL. The specified number of digestions is valid when digesting tissue up to 0.5 g following the protocol in chapter 2.2.
Storage	Upon arrival immediately store Enzyme P in aliquots at –20 °C. All other vials can be stored at 2–8 °C and have to be reconstituted before the date indicated on the box label. For information about reconstitution and storage after reconstitution of the lyophilized components refer to chapter 2.1.

1.1 Principle of the Skeletal Muscle Dissociation Kit

Mouse or rat skeletal muscle tissues can be dissociated into single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular matrix, which maintains the structural integrity of tissues.

The skeletal muscle tissue is enzymatically digested using the kit components and the gentleMACS™ Dissociator is used for the mechanical dissociation steps. After dissociation, the sample is applied to a filter to remove any remaining larger particles from the single-cell suspension.

Cells should be processed immediately for downstream applications, such as cell separation, cell culture, cellular or molecular analyses.

1.2 Background information

The Skeletal Muscle Dissociation Kit, mouse and rat has been developed for the gentle, rapid, and effective generation of single-cell suspensions from murine and rat skeletal muscle tissue. It is optimized for a high yield of viable cells, while preserving cell surface epitopes. Mature myotubes will be destroyed. The single-cell suspension can be analyzed *in vitro* for phenotype distributions, and other functional, genetic, or proteomic studies can be performed. Furthermore, dissociated cells can be subsequently cultured or isolated using MACS® Technology.

1.3 Applications

- Dissociation of mouse and rat skeletal muscle tissue into single-cell suspensions for subsequent cell separations using MACS Technology.
- Cultivation of muscle resident cell populations.
- Enumeration and phenotyping of cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- Cell culture medium without fetal bovine serum (FBS), e.g., DMEM
- MACS SmartStrainers (70 µm) (# 130-098-462)
- gentleMACS Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C
- PEB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
  Always use freshly prepared buffer. Do **not use** autoMACS Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.
  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse or rat serum albumin, mouse or rat serum, or FBS. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

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2. Protocol

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

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ Dissociation of up to 0.5 g tissue should be done in 2.5 mL; when working with 0.5–1.0 g of tissue dissociation should be done in 5 mL, scale up all reagent volumes and total volumes accordingly. If more than 1.0 g of tissue has to be digested it is recommended to use additional tubes.

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

2.1 Reagent preparation

1. Prepare Enzyme D by reconstitution of the lyophilized powder in each vial with 3 mL of DMEM. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution. For cell culture experiments subsequent to tissue dissociation, Enzyme D should be sterile filtered prior to aliquoting.
2. Enzyme P is ready to use. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months.
3. Prepare one vial of Enzyme A by reconstitution of the lyophilized powder with 1 mL Buffer A, supplied with the kit. Do not vortex. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.

   ▲ **Note:** If using the Satellite Cell Isolation Kit subsequently to skeletal muscle dissociation reconstitute the second vial of Enzyme A the same way.

2.2 Skeletal muscle dissociation protocol

1. Prepare enzyme mix by adding 2.35 mL of DMEM, 100 µL of Enzyme D, 25 µL of Enzyme P, and 18 µL of Enzyme A into a gentleMACS C Tube.
2. Cut skeletal muscle tissue into small pieces of 2–4 mm.
3. Transfer the tissue into the gentleMACS C Tube containing the enzyme mix and close it tightly.
4. (Optional) If using the heating function of the gentleMACS Octo Dissociator with Heaters attach C Tube upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters. Run program **37C_mr_SMDK_1** and continue with step 12.

   ▲ **Note:** To further increase the yield of satellite cells run program **37C_mr_SMDK_2** and continue with step 12.
5. Incubate sample for 30 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.

   ▲ **Note:** To further increase the yield of satellite cells extend the incubation time to 60 minutes at 37 °C.
6. Attach C Tube 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 rotor/stator.
7. Run the gentleMACS Program **m_muscle_01**.
8. After termination of the program, detach C Tube from the gentleMACS Dissociator.
9. Incubate sample for 30 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
10. Attach C Tube 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 rotor/stator.
11. Run again the gentleMACS Program **m_muscle_01**.
12. (Optional) Perform a short centrifugation step to collect the sample material at the tube bottom.
13. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (70 µm) placed on a 15 mL tube.

    ▲ **Note:** Dissociated tissue can be removed from the closed C Tube by pipetting through the septum-sealed opening in the center of the cap of the C Tube. Use ART 1000 REACH 1000 µL pipette tips.
14. Wash MACS SmartStrainer (70 µm) with 10 mL of DMEM.
15. Discard MACS SmartStrainer (70 µm) and centrifuge cell suspension at 300×g for 20 minutes. Aspirate supernatant completely.
16. Resuspend cells with an appropriate buffer to the required volume for further applications, for example, resuspend cells in PEB buffer for magnetic cell separation or flow cytometry.
17. (Optional) To remove erythrocytes or dead cells, use Red Blood Cell Lysis Solution (10×) (# 130-094-183), or perform a density gradient centrifugation step.

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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