Miltenyi Biotec

Adipose Tissue Dissociation Kit
mouse and rat

Order no. 130-105-808

Contents

1. Description

Components	5 vials, containing: **2 vials of Enzyme D (lyophilized powder)** **1 vial of Enzyme R (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)** **1 mL of Buffer A**
Size	For 50 digestions of 2.5 mL.
Storage	Upon arrival store all components at 2–8 °C. Reconstitute all components 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 Adipose Tissue Dissociation Kit

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

1.3 Applications

- Dissociation of mouse or rat adipose tissue into single-cell suspensions for subsequent cell separations using MACS Technology.
- Cultivation of adipose tissue resident cell populations.
- Phenotyping or enumeration of cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- DMEM (# 130-091-437)
- MACS SmartStrainers, 100 µm (# 130-098-463)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C.
- 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)
- (Optional) MACS Tissue Storage Solution (# 130-100-008)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.
- (Optional) 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).

  ▲ **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 fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

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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.

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

▲ Appropriate volume of enzyme mix based on tissue volume:

	White adipose tissue	Brown adipose tissue
Up to 0.5 g tissue	2.5 mL	1.25 mL
0.51–1.0 g tissue	5 mL	2.5 mL

If more than 1.0 g of tissue has to be digested use more tubes.

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. Prepare Enzyme R by reconstitution of the lyophilized powder in the vial with 2.7 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.

   ▲ **Note:** Make sure to thoroughly mix this suspension immediately before withdrawing the required reaction volume!
3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL of 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.

2.2 Adipose tissue dissociation protocol

1. Prepare enzyme mix by adding 2.35 mL of DMEM, 100 µL of Enzyme D, 50 µL of Enzyme R, and 12.5 µL of Enzyme A into a gentleMACS C Tube for a dissociation volume of 2.5 mL.
2. Resect the adipose tissue and cut it into small pieces of 2–4 mm. Refer to table in section 2 for the appropriate volume.
3. Transfer the tissue into the gentleMACS C Tube containing the enzyme mix and tightly close it.
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_mr_ATDK_1** and continue with step 11.

   ▲ **Note:** Close C Tube tightly beyond the first resistance.
4. Incubate sample for 20 minutes at 37 °C under continuous agitation using the MACSmix Tube Rotator.
5. 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.
6. Run the gentleMACS Program **mr_adipose_01**.
7. After termination of the program, detach C Tube from the gentleMACS Dissociator.
8. Incubate sample for 20 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
9. 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.
10. Run the gentleMACS Program **mr_adipose_01**.
11. After termination of the program, detach C Tube from the gentleMACS Dissociator.
12. (Optional) Perform a short centrifugation step up to 300×g to collect the sample material at the tube bottom.
13. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (100 µm) placed on a 15 mL or 50 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 (100 µm) with 5–10 mL of DMEM.
15. Discard the MACS SmartStrainer (100 µm) and centrifuge cell suspension at 300×g for 10 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. Process cells immediately for further applications.

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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