Miltenyi Biotec

Lung Dissociation Kit
mouse

Order no. 130-095-927

Contents

1. Description

This product is for research use only.

Components	4 vials, containing: **13 mL of Buffer S (20× Stock Solution)** **2 vials of Enzyme D (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)**
Size	For 50 digestions. The specified number of digestions is valid when digesting lung material of one mouse with an average weight of 110–150 mg following the protocol in chapter 2.2.
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 Lung Dissociation Kit

The lung tissue 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 lung tissue is enzymatically digested using the kit components, and the gentleMACS™ Dissociators are 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 Lung Dissociation Kit, mouse has been designed for the gentle, rapid, and efficient generation of single-cell suspensions from mouse lung. It is optimized for a high yield of leukocytes and endothelial cells, while preserving all cell surface epitopes. Dissociated cells can be isolated using MACS® Technology. Furthermore, the single-cell suspension can be analyzed *in vitro* for phenotype distributions, and other functional, genetic, or proteomic studies performed.

1.3 Applications

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

1.4 Reagent and instrument requirements

- PBS: phosphate-buffered saline pH 7.2
- 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.
- MACS SmartStrainers (70 µm) (# 130-098-462)
- 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) 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.

▲ Lung material of one mouse is dissociated in a volume of approximately 2.5 mL enzyme mix. The weight of the lungs of one mouse amounts to 110–150 mg (female BALB/c mouse, 6–7 weeks old).

2.1 Reagent preparation

1. Prepare 1× Buffer S by adding, for example, 1 mL of 20× Buffer S aseptically to 19 mL of sterile, distilled water. Store at 2–8 °C.

   ▲ **Note:** Handle under sterile conditions.

2. Prepare Enzyme D by reconstitution of the lyophilized powder in each vial with 3 mL of 1× Buffer S. Close the vial and wait for at least 5 minutes while inverting every minute. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. For cell culture experiments subsequent to tissue dissociation, Enzyme D should be sterile filtered prior to aliquoting. Store aliquots at –20 °C. This solution is stable for 6 months.

3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL of 1× Buffer S. 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 Lung dissociation protocol

1. Prepare enzyme mix by adding 2.4 mL of 1× Buffer S, 100 µL of Enzyme D, and 15 µL of Enzyme A into a gentleMACS C Tube.
2. (Optional) It is recommended to perfuse lungs via the right ventricle if removal of erythrocytes or circulating leukocytes is desired.
3. Dissect mouse lungs into single lobes and rinse lobes in a petri dish containing PBS, pH 7.2, to remove remaining blood.

   ▲ **Note:** Remove thymus, heart, efferent and afferent blood vessels, trachea, and connective tissue from the lung tissue.

4. Transfer lobes of the lungs of one mouse into the gentleMACS C Tube containing the enzyme mix.

   ▲ **Note:** Cell yields can be increased by injecting the enzyme into the tissue. Therefore, inflate each lung lobe on a petri dish by injecting the enzyme solution slowly 1–5-times using a 25 G needle connetcted to a 1 mL syringe.

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

   ▲ **Note:** Close C Tube tightly beyond the first resistance.

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

6. If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_m_LDK_1** and continue with step 11.
   If using the gentleMACS Dissociator without heating function run the gentleMACS Program **m_lung_01** and continue with step 7.

7. After termination of the program, detach C Tube from the gentleMACS Dissociator.

   ▲ **Note:** The lung lobes will not be completely dissociated after this step. In the unexpected event that the lobes are not dissociated at all, repeat steps 6 and 7.

8. Incubate sample for 30 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 **m_lung_02**.
11. After termination of the program, detach C Tube from the gentleMACS Dissociator.
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 2.5 mL 1× Buffer S.
15. Discard the MACS SmartStrainer (70 µm) and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
16. Resuspend cells with medium or an appropriate buffer to the required volume for further applications. For example, resuspend cells in PEB buffer for magnetic cell separatiom 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/local to find your nearest Miltenyi Biotec contact.

140-003-085.07

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