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1. Description

Components	13 mL Dissociation Buffer (20× Stock Solution) 2 vials Solution 1 (lyophilized powder) 1 vial Solution 2 (lyophilized powder)
Size	For 50 digestions. The specified number of digestions is valid when digesting a lung with an average weight of 110–150 mg following the protocol in chapter 2.2.
Storage	Store all components at 2–8 °C upon arrival. The expiration date is indicated on the kit 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 effective generation of single-cell suspensions from mouse lung. It is optimized for a high yield of leukocytes and endothelial cells, while preserving 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).
- Pre-Separation Filters, 70 µm (# 130-095-823)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C
- gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937)
- 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.

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.

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

2.1 Reagent preparation

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

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

2. Prepare Solution 1 by reconstitution of the lyophilized powder in the vial with 3 mL of 1× Dissociation Buffer. Produce aliquots of appropriate volume. Store aliquots at –20 °C. For cell culture experiments subsequent to tissue dissociation, Solution 1 should be sterile filtered prior to aliquoting. Avoid repeated freeze-thaw-cycles.

3. Prepare Solution 2 by reconstitution of the lyophilized powder in the vial with 1 mL 1× Dissociation Buffer. Do not vortex. Produce aliquots of appropriate volume. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

2.2 Lung dissociation protocol

1. Prepare enzyme mix by adding 2.4 mL of 1× Dissociation Buffer, 100 µL of Solution 1 and 15 µL of Solution 2 into a gentleMACS C Tube.

2. Dissect mouse lung and rinse lobes in a petri dish containing PBS, pH 7.2, to remove remaining blood.

▲ **Note:** Make sure to remove thymus, heart, efferent and afferent blood vessels, trachea, and connective tissue from the lung tissue.

3. Transfer lobes of one mouse lung into the gentleMACS C Tube containing the enzyme mix.

4. 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.

5. Run the gentleMACS Program **m_lung_01**.

6. 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.

7. Incubate sample for 30 minutes at 37 °C using the MACSmix Tube Rotator, or turn tube every 5 minutes to resuspend settled tissue fragments.

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

8. 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.

9. Run the gentleMACS Program **m_lung_02**.

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

11. Perform a short centrifugation step to collect the sample material at the tube bottom.

12. Resuspend sample and apply the cell suspension to a Pre-Separation Filter, 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.

13. Wash the filter with 2.5 mL 1× Dissociation Buffer.

14. Discard the filter and centrifuge cell suspension at 300×g for 10 minutes.

15. Aspirate supernatant completely and resuspend cells with 5 mL of PEB buffer.

16. (Optional) Determine cell number.

17. Centrifuge cell suspension at 300×g for 10 minutes, aspirate supernatant completely, and resuspend cells in an appropriate buffer.

18. Cells should be processed immediately for further applications.

All protocols and data sheets are available at www.miltenyibiotec.com.

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