Miltenyi Biotec

Dissociation of rat lung using the Multi Tissue Dissociation Kit 2

Contents

1. Description

1.1 Background information

Single-cell suspensions are a prerequisite for many experiments, for example to achieve the highest possible purity and recovery during cell separations with MACS® Technology. The gentleMACS™ Dissociators provide optimized programs to attain single-cell suspensions from various tissues, for example, rat lung. In combination with C Tubes, the gentleMACS Dissociators allow the automated tissue dissociation in a closed system, enabling sterile sample handling. A single tube or up to eight tubes can be processed in parallel.

This protocol has been developed to obtain single cells from rat lung using the Multi Tissue Dissociation Kit 2 in combination with the gentleMACS Dissociators.

1.2 Reagent and instrument requirements

- Multi Tissue Dissociation Kit 2 (# 130-110-203)
- Cell culture medium with fetal bovine serum (FBS)
- 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)
- (Optional) MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C.
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)

2. Protocol for the dissociation of rat lung

▲ 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.

▲ Dissociate one adult rat lung lobe in 2500 µL enzyme mix per gentleMACS C Tube. When working with whole lung material of one adult rat split the sample into two C Tubes and scale up all reagent volumes and total volumes accordingly.

▲ Operate MACSmix Tube Rotator with continuous rotation at a speed of approximately 12 rpm.

1. Harvest adult rat lung and transfer it into a 10 cm dish containing PBS. Remove blood vessels and remaining trachea from the lung tissue.
2. Dissect lung into single lobes, cut each pulmonary lobe into small pieces (2–3 mm^3), and split them equally into two fractions.
3. Add 2.3 mL of Buffer X, 62.5 µL of Enzyme P, 25 µL of Buffer Y, 100 µL of Enzyme D, and 12.5 µL of Enzyme A of the Multi Tissue Dissociation Kit 2 into a gentleMACS C Tube.

   ▲ **Note:** Do not premix Enzyme P with Enzyme D or Enzyme A.
4. Transfer each half of the tissue fraction into a separate gentleMACS C Tube containing the enzyme mix and 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 rotor/stator.
5. Run the gentleMACS Program **Multi_C_01.**
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_Multi_C** and continue with step 10.
6. After termination of the program, detach C Tube from the gentleMACS Dissociator.
7. Incubate sample for 30 minutes at 37 °C with continuous rotation using the MACSmix Tube Rotator.
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 **Multi_C_02.**
10. After termination of the program, detach C Tube from the gentleMACS Dissociator.
11. Add 7.5 mL of cell culture medium with FBS into the C Tube to stop the enzymatic reaction

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12. Resuspend sample and apply the cell suspension to a MACS® SmartStrainer (70 µm) placed on a 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.

13. Wash MACS SmartStrainer (70 µm) with 3 mL of cell culture medium with FBS.

14. Centrifuge cell suspension at 600×g for 5 minutes. Aspirate supernatant completely.

15. Resuspend cells with an appropriate buffer to the required volume for further applications.

16. (Optional) To remove erythrocytes or dead cells, use Red Blood Cell Lysis Solution (10×) (# 130-094-183), or perform a density gradient centrifugation step.

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

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