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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, mouse liver. 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 two tubes can be processed in parallel.

This protocol has been developed for the preparation of non-parenchymal mouse liver cells, i.e., liver sinusoidal endothelial cells (LSECs) and Kupffer cells, using the gentleMACS Programs `m_liver_01.02` and `m_liver_02.02`.

1.2 Reagent and instrument requirements

- gentleMACS Dissociator (# 130-093-235)
- gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- MACSmix™ Tube Rotator (# 130-090-753)
- Cell strainer (100 µm mesh size)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.
- Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- Krebs-Ringer-Buffer (KRB): 154 mM NaCl, 5.6 mM KCl, 5.5 mM Glucose, 20.1 mM HEPES, 25 mM NaHCO₃, adjust to pH 7.4 with NaOH.
- 0.5 M CaCl₂ solution
- 0.2 M MgCl₂ solution
- Collagenase IV solution: Prepare a solution containing 5000 Collagenase Digestion Units (CDU)/mL in KRB (e.g. Collagenase IV C5138, Sigma-Aldrich).

▲ **Note:** Please refer to manufacturer's specification for lot specific activity of Collagenase IV.

- DNase I solution: Prepare a solution containing 30,000 U/mL DNase I in KRB (e.g. using DNase I, AppliChem).
- 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).

2. Protocol for the preparation of non-parenchymal mouse liver cells with Collagenase IV treatment

▲ 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.

▲ The weight of one mouse liver amounts to 750–1200 mg (CD1 mouse, 8 weeks old).

▲ One mouse liver can be dissociated per C Tube.

1. Prepare dissociation mix by pipetting 4.4 mL KRB into a gentleMACS C Tube. Add 20 µL CaCl₂ solution, 50 µL MgCl₂ solution, 500 µL Collagenase IV solution (final concentration: 500 CDU/mL), and 25 µL DNase I solution (final concentration: 150 U/mL).

▲ **Note:** For optimum performance of Collagenase IV, the dissociation mix should be prepared on the day of the experiment.

2. Incubate dissociation mix for 30 minutes at 37 °C.

3. Rinse liver with KRB.

▲ **Note:** Remove gallbladder with forceps before dissecting the mouse liver. Resect connective tissue.

4. Transfer the liver into the C Tube containing prewarmed dissociation mix.

5. 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.

6. Run the gentleMACS Program `m_liver_01.02`.

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

8. Incubate sample for 30 minutes at 37 °C under slow continuous rotation using the MACSmix Tube Rotator.

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

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_liver_02.02`.

11. After termination of the program, detach C Tube from the gentleMACS Dissociator.
12. (Optional) Perform a short centrifugation step to collect sample at the tube bottom.
13. Rinse a 50 mL tube and a cell strainer (100 µm mesh size) with PEB buffer. Discard PEB buffer and place pre-wet cell strainer onto the tube.
14. Resuspend sample in the C Tube and apply it onto the cell strainer. Rinse C Tube with 5 mL PEB buffer and apply to cell strainer. Wash cell strainer with additional 10 mL of PEB buffer.
▲ 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.
15. Discard cell strainer and add 10 mL of PEB buffer to the collected sample.
16. Centrifuge cell suspension at 17–21×g for 4 minutes at 4 °C to remove contaminating hepatocytes.
17. Collect supernatant as fraction with the target cells by using a serological pipette and transfer to a new 50 mL tube rinsed with PEB buffer.
▲ Note: Do not decant supernatant.
18. Centrifuge cell suspension at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.
19. Resuspend cell pellet in 1 mL PEB buffer and add 10 mL 1× Red Blood Cell Lysis Solution.
▲ Note: For preparation of 1× Red Blood Cell Lysis Solution, refer to the data sheet of Red Blood Cell Lysis Solution (10×) (# 130-094-183).
20. Incubate for 5 minutes at room temperature.
21. Add 30 mL of PEB buffer and centrifuge at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.
22. Resuspend cell pellet in 1–2 mL PEB buffer and adjust volume with PEB buffer to a final volume of 30 mL.
23. Centrifuge cell suspension at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.
24. Resuspend cells with PEB buffer to the required volume for further applications.

All gentleMACS Protocols are available at www.miltenyibiotec.com.

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