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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 heart. 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 to obtain endothelial cells and Sca-1⁺ cells from mouse heart using the gentleMACS Programs *m_heart_01* and *m_heart_02*. Subsets of Sca-1⁺ cells have been designated as progenitor/stem cells in mouse heart.^{1,2}

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 (70 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)
- Hanks' Balanced Salt Solution with Ca²⁺ and Mg²⁺ (HBSS): 1.26 mM CaCl₂, 0.49 mM MgCl₂•6H₂O, 0.41 mM MgSO₄•7H₂O, 5.3 mM KCl, 0.44 mM KH₂PO₄, 4.17 mM NaHCO₃, 137.93 mM NaCl, 0.34 mM Na₂HPO₄, 5.55 mM Dextrose. Keep buffer cold (2–8 °C).
- Collagenase II solution: Prepare a solution with 10,000 U/mL Collagenase II (Worthington, CLS-2) in HBSS.
- DNase I solution: Prepare a solution containing 30,000 U/mL DNase I (e.g. 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 dissociation of mouse heart with Collagenase II 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 heart amounts to 80–115 mg (female BALB/c mouse, 6–7 weeks old).
- ▲ One mouse heart or up to four mouse hearts (maximal 400 mg) can be processed per C Tube.

1. Cut mouse heart transversely in two halves and rinse with cold HBSS.
2. Transfer mouse heart into the gentleMACS C Tube containing 4.7 mL of HBSS. Add 300 µL Collagenase II solution (final Collagenase II concentration: 600 U/mL) and 10 µL DNase I solution (final DNase I concentration: 60 U/mL).
 - ▲ **Note:** This protocol has been optimized for the use of Collagenase II (Worthington, CLS-2). The use of other enzymes or enzyme concentrations is not recommended.
3. 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.
4. Run the gentleMACS Program *m_heart_01*.
5. After termination of the program, detach C Tube from the gentleMACS Dissociator.
6. 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.
7. 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.
8. Run the gentleMACS Program *m_heart_02*.
9. After termination of the program, detach C Tube from the gentleMACS Dissociator.
10. Perform a short centrifugation step to collect the sample material at the tube bottom.
11. Resuspend sample and apply the cell suspension to a cell strainer (70 µm mesh size) 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.
12. Wash the cell strainer with 5 mL of HBSS.

13. Discard cell strainer and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
14. 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).
15. Incubate for **maximal 2 minutes** at room temperature.
16. Centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
17. Resuspend cells in 1 mL PEB buffer and add to a final volume of 10 mL.
18. Centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
19. Resuspend cells with PEB buffer to the required volume for further applications.

3. References

1. Oh, H. *et al.* (2003) Cardiac progenitor cells from adult myocardium: Homing, differentiation, and fusion after infarction. *Proc. Natl. Acad. Sci. USA* 100: 12313–12318.
2. Pfister, O. *et al.* (2005) CD31⁻ but Not CD31⁺ Cardiac Side Population Cells Exhibit Functional Cardiomyogenic Differentiation. *Circ. Res.* 97: 52–61.

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

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