Miltenyi Biotec

Dissociation of adult rat heart 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 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 up to eight tubes can be processed in parallel.

This protocol has been developed to obtain single cells from adult rat heart using the Multi Tissue Dissociation Kit 2 in combination with the gentleMACS Dissociators. It is optimized for a high yield of viable non-myocytes, e.g., cardiac fibroblasts and endothelial cells. The single-cell suspension can be cultured and utilized for functional, genetic, or molecular studies.

1.2 Reagent and instrument requirements

- Multi Tissue Dissociation Kit 2 (# 130-110-203)
- Phosphate-buffered saline (PBS), pH 7.4
- Cell culture medium without fetal bovine serum (FBS), e.g., RPMI 1640 or DMEM
- Cell culture medium with 20% 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)
- 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.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as rat serum albumin, rat serum, or FBS. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

Additional requirements for debris removal and red blood cell lysis

- Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- Debris Removal Solution (# 130-109-398)
- Centrifuge with a swinging bucket rotor
- 15 mL reagent 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.

▲ Volumes given below are for 1 adult rat heart per C Tube and 2.5 mL of enzyme mix.

2.1 Adult rat heart dissociation protocol

1. Prepare enzyme mix of the Multi Tissue Dissociation Kit 2 according to table below.

Enzyme mix 1		Enzyme mix 2		
Enzyme P	Buffer X	Buffer Y	Enzyme A	Enzyme D
62.5 µL	2300 µL	25 µL	12.5 µL	100 µL

2. Harvest adult rat heart and transfer into a 10 cm dish containing PBS. Cut vessels and remaining connective tissue away from the ventricles. Cut heart into small pieces (1–2 mm^3).
3. Preheat enzyme mix 1 for 5 minutes at 37 °C.

   ▲ **Note:** Preheating is not required if using the heating function of the gentleMACS Octo Dissociator with Heaters.
4. Add 2362.5 µL of enzyme mix 1 to 137.5 µL of enzyme mix 2.
5. Transfer harvested tissue into the gentleMACS C Tube.

   ▲ **Note:** To reduce the volume of washing medium within the tube let tissue settle down by gravity and remove supernatant carefully.
6. Add 2.5 mL of enzyme mix, tightly close the C Tube.

   ▲ **Note:** Close C Tube tightly beyond the first resistance.
7. Invert C Tube and place it with the cap down. To maximize cell recovery the C Tube should remain in this orientation until step 12.
8. (Optional) If using the heating function of the gentleMACS Octo Dissociator with Heaters attach C Tube upside down onto

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the sleeve of the gentleMACS™ Octo Dissociator with Heaters. Run program **37C_Multi_G** and continue with step 13.

9. Incubate sample without agitation for 15 minutes at 37 °C.
10. Attach C Tube onto the sleeve of the gentleMACS Dissociator.
11. Run the gentleMACS Program **Multi_G**.
12. Repeat steps 9–11 two times.
13. After termination of the program, detach C Tube from the gentleMACS Dissociator and add 7.5 mL of cell culture medium with 20% FBS.
14. Resuspend sample and apply the cell suspension to a MACS® SmartStrainer (70 µm) placed on a suitable tube.
15. Wash MACS SmartStrainer (70 µm) with 3 mL of cell culture medium with 20% FBS.
16. Discard filter and centrifuge cell suspension at 600×g for 5 minutes. Aspirate supernatant completely.
17. Proceed with debris removal (refer to section 2.2).

2.2 Debris removal

▲ Always use pre-cooled buffers and solutions (4 °C).

1. Resuspend cell suspension carefully with 6200 µL of cold PBS and transfer cell suspension to a 15 mL tube. Do not vortex.
2. Add 1800 µL of cold Debris Removal Solution.
3. Mix well by pipetting 10 times slowly up and down using a 5 mL pipette.
4. Overlay very gently with 4 mL of cold PBS.

   ▲ **Note:** Tilt tube and pipette very slowly to ensure that the PBS phase overlays the cell suspension and phases are not mixed.
5. Centrifuge at 4 °C and 3000×g for 10 minutes with full acceleration and full brake. Three phases are formed.
6. Aspirate the two top phases completely and discard them.
7. Fill up with cold PBS to a final volume of 15 mL.
8. Gently invert the tube three times. Do not vortex!
9. Centrifuge at 4 °C and 1000×g for 10 minutes with full acceleration and full brake.
10. Aspirate supernatant completely.
11. Resuspend cells carefully in the appropriate buffer or medium by pipetting slowly up and down. Do not vortex!
12. Proceed with red blood cell lysis (refer to section 2.3).

2.3 Red blood cell lysis

1. Resuspend cell pellet in 1 mL of PEB buffer and add 10 mL of 1× Red Blood Cell Lysis Solution to remove erythrocytes.
2. Incubate for maximal 2 minutes at room temperature (19–25 °C).
3. Centrifuge at 600×g for 5 minutes. Aspirate supernatant completely.
4. Add 15 µL of Enzyme A of the Multi Tissue Dissociation Kit 2 to 10 mL PBS in a fresh tube.
5. Resuspend cell pellet in 10 mL PBS containing Enzyme A from step 4.
6. Centrifuge at 600×g for 5 minutes. Aspirate supernatant completely.
7. Resuspend cells with appropriate buffer or medium to the required volume for further applications.

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

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