Miltenyi Biotec

Dissociation of mouse kidney 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, mouse kidney. 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 mouse kidney using the Multi Tissue Dissociation Kit 2 in combination with the gentleMACS Dissociators.
Please note that following antigens may be epitope-sensitive when using the Multi Tissue Dissociation Kit 2, which can result in reduced detection levels: CD8a, CD19, CD45R (B220), CD25, CD44, CD69, NK1.1, Ter119.

1.2 Reagent and instrument requirements

- Multi Tissue Dissociation Kit 2 (# 130-110-203)
- Phosphate-buffered saline (PBS), pH 7.4
- MACS SmartStrainers (100 µm) (# 130-098-463)
- 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 mouse kidney

▲ 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 up to 1 g tissue in ~5 mL enzyme mix per gentleMACS C Tube.

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

1. Harvest mouse kidneys and transfer into a 10 cm dish. Utilizing forceps, carefully remove renal capsules.
2. Add 4.8 mL of Buffer X, 50 µL of Enzyme P, 50 µL of Buffer Y, 100 µL of Enzyme D, and 20 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.
3. Quarter mouse kidneys and transfer the tissue 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:** It has to be ensured that the sample material is located in the area of the rotor/stator.
5. If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_Multi_E** and continue with step 10. If using the gentleMACS Dissociator without heating function run the gentleMACS Program **Multi_E_01** and continue with step 6.
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_E_02.**
10. After termination of the program, detach C Tube from the gentleMACS Dissociator.
11. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (100 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.

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12. Wash MACS® SmartStrainer (100 µm) with 15 mL of PBS.
13. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
14. Resuspend cells with an appropriate buffer to the required volume for further applications.
15. (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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