Miltenyi Biotec

Dissociation of human kidney using the Multi Tissue Dissociation Kit 1

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, human 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 human kidney using the Multi Tissue Dissociation Kit 1 in combination with the gentleMACS Dissociators.

1.2 Reagent and instrument requirements

- Multi Tissue Dissociation Kit 1 (# 130-110-201)
- RPMI 1640 or DMEM
- 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 human 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 0.5 g tissue in ~2.5 mL enzyme mix per gentleMACS C Tube. When working with of 0.51–1.0 g tissue, use 5 mL enzyme mix per tube. A maximum of 1 g tissue per C Tube can be processed.

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

1. Prepare enzyme mix by adding 2.35 mL of serum-free RPMI 1640 or DMEM, 100 µL of Enzyme D, 50 µL of Enzyme R, and 12.5 µL of Enzyme A of the Multi Tissue Dissociation Kit 1 into a gentleMACS C Tube for up to 0.5 g of tissue.
2. Cut the human kidney into small pieces of 2–4 mm.
3. 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. Run the gentleMACS Program **Multi_B.**
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_Multi_B** and continue with step 14.
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_B.**
10. After termination of the program, detach C Tube from the gentleMACS Dissociator.
11. Incubate sample for 30 minutes at 37 °C with continuous rotation using the MACSmix Tube Rotator.
12. 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.

SP0086.01

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 877 591 1060
macs@miltenyibiotec.com

13. Run the gentleMACS™ Program **Multi_B.**
14. After termination of the program, detach C Tube from the gentleMACS Dissociator.
15. 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.
16. Wash MACS SmartStrainer (70 µm) with 15 mL of RPMI 1640 or DMEM.
17. Centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant completely.
18. Resuspend cells with an appropriate buffer to the required volume for further applications.
19. (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.**

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.