

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Reagent and instrument requirements
2. Protocol for the dissociation of mouse ear

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 ear. 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 such as T cells, dendritic cells, and macrophages from mouse ear using the Multi Tissue Dissociation Kit 1 in combination with the gentleMACS Dissociators.

1.2 Applications

- Obtain single-cell suspension for subsequent flow cytometric analysis using REAfinity™ Antibodies or flow sorting.
- Isolation of T cells, dendritic cells, and macrophages using MACS Technology, e.g., CD45 MicroBeads, from single-cell suspension.

1.3 Reagent and instrument requirements

- Multi Tissue Dissociation Kit 1 (# 130-110-201)
- RPMI 1640 or DMEM
- 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.

2. Protocol for the dissociation of mouse ear

▲ 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 4 mouse ears in ~2.5 mL enzyme mix per gentleMACS C Tube. When working with up to 8 ears, use 5 mL enzyme mix per tube. Do not use more than 5 mL enzyme mix per C Tube.

▲ 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.
2. Separate the internal and external faces of the mouse ears by using forceps.
3. Cut the separated mouse ear halves into small pieces of 2–4 mm.
4. Transfer the tissue into the gentleMACS C Tube containing the enzyme mix and tightly close it.
If using the heating function of the gentleMACS Octo Dissociator with Heaters attach C Tube upside down onto the sleeve of the gentleMACS Dissociator, run program **37C_Multi_H**, and continue with step 9.
5. Incubate sample for 90 minutes at 37 °C with continuous rotation using, e.g., the MACSmix Tube Rotator.
6. 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.
7. Run the gentleMACS Program **Multi_H**.
8. After termination of the program, detach C Tube from the gentleMACS Dissociator.
9. (Optional) After termination of the program, detach C Tube from the gentleMACS Dissociator and perform a short spin up to 300×g to collect the sample at the bottom of the tube.
10. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (100 µm) placed on a 15 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. To prevent cartilage pieces clogging the tip, proceed a short spin of the C Tube and pipette carefully.
11. Wash MACS SmartStrainer (100 µm) with 5–10 mL of RPMI 1640 or DMEM.
12. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

13. Resuspend cells with an appropriate buffer to the required volume for further applications, for example, cell separation using MACS® Technology.

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.

gentleMACS, MACS, the MACS logo, MACSmix, and REAfinity are registered trademarks or trademarks of Miltenyi Biotec GmbH and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Copyright © 2017 Miltenyi Biotec GmbH and/or its affiliates. All rights reserved.