

## Contents

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

## 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 embryo. 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 fibroblasts from mouse embryo using the Multi Tissue Dissociation Kit 3 in combination with the gentleMACS Dissociators.

### 1.2 Reagent and instrument requirements

- Multi Tissue Dissociation Kit 3 (# 130-110-204)
- DMEM with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin
- 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.

## 2. Protocol for the dissociation of mouse embryo

- ▲ 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 embryo in 1 mL enzyme mix per gentleMACS C Tube.
  - ▲ Operate MACSmix Tube Rotator with continuous rotation at a speed of approximately 12 rpm.
1. Add 0.9 mL of Buffer X and 100 µL of Enzyme T of the Multi Tissue Dissociation Kit 3 into a 1.5 mL tube.
  2. Harvest mouse embryos.  
If using the heating function of the gentleMACS Octo Dissociator with Heaters continue with step 5.
  3. Transfer one embryo into the 1.5 mL tube containing the enzyme mix and tightly close it.
  4. Incubate sample for 10 minutes at 37 °C with continuous rotation using the MACSmix Tube Rotator. Continue directly with step 9.
  5. Transfer one embryo into a gentleMACS C Tube containing the enzyme mix and tightly close it.
  6. Attach C Tube upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters.  
▲ **Note:** It has to be ensured that the sample material is located in the area of the rotor/stator.
  7. Run the gentleMACS Program **37C\_Multi\_D**.
  8. After termination of the program, detach C Tube from the gentleMACS Octo Dissociator with Heaters.
  9. Add 3 mL of DMEM with 10% FBS and 1% penicillin/streptomycin and mix.
  10. 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.
  11. Run the gentleMACS Program **Multi\_D**.
  12. (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.
  13. 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.

14. Wash MACS® SmartStrainer (100 µm) with 15 mL of DMEM with 10% FBS and 1% penicillin/streptomycin.
15. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
16. Resuspend cells with an appropriate buffer to the required volume for further applications.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

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