

Dissociation of mouse embryo using the Multi Tissue Dissociation Kit 3

Contents

1. Description

1.1 Background information

1.2 Reagent and instrument requirements

2. Protocol for the dissociation of mouse embryo

1. Description

This product is for research use only.

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)
- gentleMACS™ Octo Dissociator with Heaters or gentleMACS Dissociator in combination with a MACSmix™ Tube Rotator (# 130 090 753) and an incubator at +37 °C
- gentleMACS C Tubes (# 130-093-237)
- MACS SmartStrainers (100 µm) (# 130-098-463)
- DMEM with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin
- Phosphate-buffered saline (PBS), pH 7.4

2. Protocol for the dissociation of mouse embryo

▲ For details on the use of gentleMACS Dissociators, refer to the respective user manual and www.miltenyibiotec.com/gentlemacs.

▲ 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 970 µL of Buffer X and 30 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.
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.

Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

gentleMACS, MACS, MACSmix, and the Miltenyi Biotec logo are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG 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 © 2024 Miltenyi Biotec and/or its affiliates. All rights reserved.