Miltenyi Biotec

Liver Dissociation Kit
mouse

Order no. 130-105-807

Contents

1. Description

Components	4 vials, containing: **2 vials of Enzyme D (lyophilized powder)** **1 vial of Enzyme R (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)**
Size	For 25 digestions. The specified number of digestions is valid when digesting a liver that do not exceed 1.2 g in weight following the protocol in chapter 2.2.
Storage	Upon arrival store all components at 2–8 °C. Reconstitute all components before the date indicated on the box label. For information about reconstitution and storage after reconstitution of the lyophilized components refer to chapter 2.1.

1.1 Principle of the Liver Dissociation Kit

Mouse liver can be dissociated into single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular matrix, which maintains the structural integrity of tissues.

The mouse liver is enzymatically digested using the kit components and the gentleMACS™ Dissociators are used for the mechanical dissociation steps. After dissociation, the sample is applied to a filter to remove any remaining larger particles from the single-cell suspension.

Cells should be processed immediately for downstream applications, such as cell separation, cell culture, cellular or molecular analyses.

1.2 Background information

The Liver Dissociation Kit, mouse has been developed for the gentle, rapid, and efficient generation of single-cell suspensions from mouse liver. It is optimized for a high yield of non-parenchymal mouse liver cells, i.e., liver sinusoidal endothelial cells (LSECs) and Kupffer cells, while preserving most cell surface epitopes. Dissociated cells can be subsequently cultured or isolated using MACS® Technology. Furthermore, the single cell suspensions can be analyzed for phenotype distributions and other functional, genetic, or proteomic studies can be performed.

1.3 Applications

- Dissociation of mouse liver for subsequent cell separations using MACS Technology.
- Cultivation of LSECS or Kupffer cells.
- Phenotyping or enumeration of cells by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- PEB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
- DMEM with stable glutamine
- 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.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

1. Protocol

▲ 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.

▲ The weight of one mouse liver amounts to 750–1200 mg (CD1, C57BL/6, or BALB/c mouse, 8 weeks old).

▲ Operate MACSmix Tube Rotator on permanent run at a speed of approximately 12 rpm.

2.1 Reagent preparation

1. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL DMEM with stable glutamine. Do not try to resuspend by pipetting or vortexing. Invert vial after closing and wait 5–10 minutes to dissolve the pellet. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution. For cell culture experiments subsequent to tissue dissociation, Enzyme D should be sterile filtered prior to aliquoting.
2. Prepare Enzyme R by reconstitution of the lyophilized powder in the vial with 2.7 mL of DMEM with stable glutamine. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.

   ▲ **Note:** Make sure to thoroughly mix this suspension immediately before withdrawing the required reaction volume!
3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL DMEM with stable glutamine. Do not vortex. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.

2.2 Liver dissociation protocol

1. Prepare dissociation mix by pipetting 4.7 mL DMEM into a gentleMACS™ C Tube. Add 200 µL Enzyme D solution, 100 µL Enzyme R solution, and 20 µL Enzyme A solution.
   If using the heating function of the gentleMACS Octo Dissociator with Heaters, continue directly with step 3.
2. Incubate dissociation mix for 30 minutes at 37 °C in an incubator or 15 minutes in a water bath.
3. Rinse liver with DMEM.

   ▲ **Note:** Remove gallbladder with forceps before dissecting the mouse liver. Resect connective tissue.
4. Transfer liver into the C Tube containing the dissociation mix.
5. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.

   ▲ **Note:** Close C Tube tightly beyond the first resistance.

   ▲ **Note:** It has to be ensured that the sample material is located in the area of the rotor/stator.
6. Run the gentleMACS Program **m_liver_03**.
   If using the heater function of the gentleMACS Octo Dissociator with Heaters, run program **37C_m_LIDK_1** and continue with step 11.
7. After termination of the program, detach C Tube from the gentleMACS Dissociator.
8. Incubate sample for 30 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
9. 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.
10. Run the gentleMACS Program **m_liver_04**.
11. After termination of the program, detach C Tube from the gentleMACS Dissociator.
12. Resuspend sample and apply the cell suspension to a MACS® SmartStrainer (100 µm).

    ▲ **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.
13. Wash the filter with 5 mL DMEM with stable glutamine.

    ▲ **Note:** For maximum cell recovery, rinse the used C Tube with wash buffer before transfer to filter.
14. Discard the filter and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
15. Resuspend cells with PEB buffer to the required volume for further applications.
16. Process cells immediately 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.

140-004-706.04

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

autoMACS, gentleMACS, MACS, MACSmix, and the Miltenyi Biotec logo are registered trademarks or trademarks of Miltenyi Biotec 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.