Miltenyi Biotec

Liver Dissociation Kit
mouse

Order no. 130-105-807

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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 to +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

- gentleMACS Octo Dissociator with Heaters or gentleMACS Dissociator with MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at +37 °C.
- gentleMACS C Tubes (# 130-093-237)
- MACS SmartStrainers (100 µm) (# 130-098-463)
- 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 to +8 °C).
- Sterile, deionized water
- DMEM with stable glutamine

2. Protocol

▲ 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.

▲ 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!

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3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL sterile, deionized water. 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 with stable glutamine 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 with stable glutamine.
   ▲ **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).
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.

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