Miltenyi Biotec

Tumor Dissociation Kit
mouse

Order no. 130-096-730

Contents

1. Description

This product is for research use only.

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 50 digestions. The specified number of digestions is valid when digesting a tumor in a range of 0.04–1 g following the protocol in chapter 2.2.
Storage	Upon arrival immediately store all components at +2 to +8 °C. Reconstitute all components before the date indicated on the kit box label. For information about reconstitution and storage after reconstitution of the lyophilized components refer to chapter 2.1.

1.1 Principle of the Tumor Dissociation Kit, mouse

Tumor tissues 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 tumor tissue 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 Tumor Dissociation Kit, mouse has been developed for the gentle, rapid, and effective generation of single-cell suspensions from implanted or induced mouse tumor tissue. It is optimized for a high yield of tumor cells, stromal cells, and tumor infiltrating lymphocytes (TILs), while preserving cell surface epitopes. For detailed information about marker preservation, please contact Technical Support at technicalsupport@miltenyi.com.
Dissociated cells can be subsequently cultured or isolated using MACS® Technology. Furthermore, the single-cell suspension can be analyzed for phenotype distributions, and other functional, genetic, or proteomic studies can be performed.

1.3 Applications

- Dissociation of mouse tumor tissue into single-cell suspensions for subsequent cell separations using MACS Technology.
- Cultivation of tumor cell or TIL populations.
- Phenotyping or enumeration of tumor cell or TIL populations 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 (30 µm) (# 130-098-458) or MACS SmartStrainers (70 µm) (# 130-098-462)
- Sterile, deionized water
- RPMI 1640 or DMEM
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- (Optional) Dead Cell Removal Kit (# 130-090-101)
- (Optional) Debris Removal Solution (# 130-109-398)
- (Optional) MACS Tissue Storage Solution (# 130-100-008)

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2. Protocols

▲ 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.

▲ Tumor tissue in a range of 0.04–1 g is dissociated in a volume of approximately 2.5 mL enzyme mix.

▲ The MACSmix Tube Rotator is used with slow, continuous rotation.

2.1 Reagent preparation

1. Prepare Enzyme D by reconstitution of the lyophilized powder in each vial with 3 mL of RPMI 1640 or DMEM. 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 RPMI 1640 or DMEM. 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 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 Tumor dissociation protocols

▲ Tumor tissue can be classified as soft, medium, or tough depending on the histological composition of the tissue. For some examples of mouse tumor types refer to the table below.

Tumor type	Tumor examples
Soft/medium	Melanoma (induced by B16 cell line) or colon (induced by CT26 cell line)
Tough	Breast (induced by 4T1 cell line) or lung (induced by TC1 cell line)

1. Prepare enzyme mix by adding 2.35 mL of RPMI 1640 or DMEM, 100 µL of Enzyme D, 50 µL of Enzyme R, and 12.5 µL of Enzyme A into a gentleMACS C Tube.

   ▲ **Note:** For the analysis of tumor infiltrating leukocytes (TILs) it is recommend to reduce the content of enzyme R to 20% in the enzyme mix (e.g., add 10 µL of enzyme R). Reducing the content of enzyme R will help to preserve cell surface epitopes but may lead to lower cell yields and viability of endothelial cells, epithelial cells, and tumor-associated fibroblasts (TAFs).
2. Remove fat, fibrous and necrotic areas from the tumor sample.
3. Cut the tumor into small pieces of 2–4 mm.
4. Transfer the tissue into the gentleMACS C Tube containing the enzyme mix.
5. Tightly close C Tube and attach it 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.

2.2.1 Dissociation using the gentleMACS™ Octo Dissociator with Heaters

1. Choose an appropriate gentleMACS™ Program according to the table below.

Tumor type	gentleMACS Program
Soft/medium	37C_m_TDK_1
Tough	37C_m_TDK_2

2. Run the selected program and continue with section 2.2.2, step 6.

2.2.2 Dissociation using the gentleMACS™ Dissociator or the gentleMACS Octo Dissociator

1. Start the **dissociation of either soft, medium, or tough tumors** by running the gentleMACS program **m_impTumor_02.**
2. After termination of the program, detach C Tube from the gentleMACS Dissociator.
3. Incubate sample for 40 minutes at +37 °C under continuous rotation using the MACSmix™ Tube Rotator.
4. 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.
5. Run the next gentleMACS Program according to the table below.

Tumor type	gentleMACS Program
Soft/medium	1× m_impTumor_03
Tough	2× m_impTumor_03

6. After termination of the program, detach C Tube from the gentleMACS Dissociator.

   ▲ **Note:** When working with tough tumors, some larger pieces of tissue may remain. To further increase the cell yield, allow the remaining tissue to settle and transfer the supernatant to a new tube. Add 4 mL RPMI 1640 or DMEM to the C Tube with the remaining tissue pieces. Insert tube onto the sleeve of the gentleMACS Dissociator. Run program **m_impTumor_01**. Combine the resulting cell suspension with the previously removed supernatant.
7. (Optional) Perform a short centrifugation step to collect the sample material at the bottom of the tube.
8. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (30 µm or 70 µm) placed on a 15 mL tube.
9. Wash cell MACS SmartStrainer (30 µm or 70 µm) with 10 mL of RPMI 1640 or DMEM.
10. Centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant completely.
11. (Optional) If sample appears 'viscous' after dissociation (released DNA), perform a DNase treatment: Centrifuge cell suspension 300×g for 5 minutes, remove supernatant completely, and resuspend pellet in 5 mL RPMI 1640 or DMEM. Add 200 U/mL DNase and incubate for 5 minutes at room temperature. Wash 300×g for 5 minutes with 5 mL serum-containing buffer. Remove supernatant completely and resuspend in RPMI 1640 or DMEM.
12. Resuspend cells as required for further applications.

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13. (Optional) To remove erythrocytes or dead cells, use Red Blood Cell Lysis Solution (10×) (# 130-094-183) or the Dead Cell Removal Kit (# 130-090-101).
14. (Optional) In case of excess of debris, use Debris Removal Solution (# 130-109-398).

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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