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1. Description

Components	2 vials of Solution 1 (lyophilized powder) 1 vial of Solution 2 (lyophilized powder) 1 vial of Solution 3 (lyophilized powder) 1 mL of Reconstitution Buffer for Solution 3
Size	For 25 digestions. The specified number of digestions is valid when digesting a tumor in a range of 0.01–2 g following the protocol in chapter 2.2.
Storage	Store all components at 2–8 °C upon arrival. Reconstitute all components before 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 Tumor Dissociation Kit

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, human has been developed for the gentle, rapid, and effective generation of single-cell suspensions from primary human tumor tissue or xenografts. It is optimized for a high yield of tumor cells and tumor infiltrating lymphocytes (TILs), while preserving cell surface epitopes. The single-cell suspension can be analyzed *in vitro* for phenotype distributions, and other functional, genetic, or proteomic studies performed. Furthermore, dissociated cells can be subsequently cultured or isolated using MACS® Technology.

1.3 Applications

- Dissociation of primary human tumor tissue or xenografts 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

- (Optional) 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).
 - ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- RPMI 1640 (# 130-091-440)
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- Cell strainer (70 µm mesh)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C.
- gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

2. 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.

▲ Tumor tissue in a range of 0.01–2 g is dissociated in a volume of approximately 5 mL enzyme mix.

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

2.1 Reagent preparation

1. Prepare Solution 1 by reconstitution of the lyophilized powder in each vial with 3 mL of RPMI 1640. Prepare aliquots of appropriate volume and 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, Solution 1 should be sterile filtered prior to aliquoting.

2. Prepare Solution 2 by reconstitution of the lyophilized powder in the vial with 2.7 mL RPMI 1640. Prepare aliquots of appropriate volume and 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 Solution 3 by reconstitution of the lyophilized powder in the vial with 1 mL Reconstitution Buffer for Solution 3 supplied with the kit. Do not vortex. Prepare aliquots of appropriate volume and avoid repeated freeze-thaw-cycles. Store aliquots at -20°C . This solution is stable for 6 months after reconstitution.

2.2 Tumor dissociation protocols

2.2.1 Dissociation of soft tumors

▲ For example, dissociation of melanoma, ovarian cancer, colon cancer, and hypopharyngeal cancer.

1. Prepare enzyme mix by adding 4.7 mL of RPMI 1640, 200 μL of Solution 1, 100 μL of Solution 2, and 25 μL of Solution 3 into a gentleMACS C Tube.

2. Cut the tumor into small pieces of 2–4 mm.

▲ **Note:** Remove fat, fibrous and necrotic areas from the tumor sample.

3. Transfer the tissue pieces into the gentleMACS C Tube containing the enzyme mix.

4. 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.

5. Run the gentleMACS Program **h_tumor_01**.

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

7. Incubate sample for 30 minutes at 37°C under continuous rotation using the MACSmix Tube Rotator.

8. 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.

9. Run the gentleMACS Program **h_tumor_02**.

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

11. Incubate sample for 30 minutes at 37°C under continuous rotation using the MACSmix Tube Rotator.

12. 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.

13. Run the gentleMACS Program **h_tumor_03**.

14. (Optional) Perform a short centrifugation step to collect the sample material at the bottom of the tube.

15. Resuspend sample and apply the cell suspension to an appropriate cell strainer 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.

16. Wash cell strainer with 20 mL of RPMI 1640.

17. Centrifuge cell suspension at $300\times g$ for 7 minutes. Aspirate supernatant completely.

18. Resuspend cells as required for further applications.

19. (Optional) To remove erythrocytes or dead cells, use Red Blood Cell Lysis Solution (10 \times) (# 130-094-183), or perform a density gradient centrifugation step.

2.2.2 Dissociation of medium tumors

▲ For example, dissociation of lung adenocarcinoma and prostate cancer.

1. Prepare enzyme mix by adding 4.7 mL of RPMI 1640, 200 μL of Solution 1, 100 μL of Solution 2, and 25 μL of Solution 3 into a gentleMACS C Tube.

2. Cut the tumor into small pieces of 2–4 mm.

▲ **Note:** Remove fat, fibrous and necrotic areas from the tumor sample.

3. Transfer the tissue pieces into the gentleMACS C Tube containing the enzyme mix.

4. 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.

5. Run the gentleMACS Program **h_tumor_01**.

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

7. Incubate sample for 30 minutes at 37°C under continuous rotation using the MACSmix Tube Rotator.

8. 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.

9. Run the gentleMACS Program **h_tumor_02**.

10. After termination of the program, detach C Tube from the gentleMACS Dissociator.
 11. Incubate sample for 30 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
 12. 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.
 13. Run the gentleMACS Program **h_tumor_02**.
 14. (Optional) Perform a short centrifugation step to collect the sample material at the bottom of the tube.
 15. Resuspend sample and apply the cell suspension to an appropriate cell strainer 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.
 16. Wash cell strainer with 20 mL of RPMI 1640.
 17. Centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant completely.
 18. Resuspend cells as required for further applications.
 19. (Optional) To remove erythrocytes or dead cells, use Red Blood Cell Lysis Solution (10×) (# 130-094-183), or perform a density gradient centrifugation step.
11. Incubate sample for 30 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
 12. 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.
 13. Run the gentleMACS Program **h_tumor_01**.
 14. (Optional) Some larger pieces of tissue may remain. To further increase the cell yield allow the remaining tissue to settle and remove 80% (4 mL) of the supernatant to a fresh tube. Insert the C-tube with the remaining tissue pieces onto the sleeve of the gentleMACS separator and run program **m_imptumor_01**. Combine the resulting cell suspension with the previously removed supernatant.
 15. Resuspend sample and apply the cell suspension to an appropriate cell strainer 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.
 16. Wash cell strainer with 20 mL of RPMI 1640.
 17. Centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant completely.
 18. Resuspend cells as required for further applications.
 19. (Optional) To remove erythrocytes or dead cells, use Red Blood Cell Lysis Solution (10×) (# 130-094-183), or perform a density gradient centrifugation step.

2.2.3 Dissociation of tough tumors

▲ For example, dissociation of breast cancer, pancreatic cancer, hepatocellular carcinoma as well as head and neck squamous cell carcinoma.

1. Prepare enzyme mix by adding 4.7 mL of RPMI 1640, 200 µL of Solution 1, 100 µL of Solution 2, and 25 µL of Solution 3 into a gentleMACS C Tube.
2. Cut the tumor into small pieces of 2–4 mm.
▲ **Note:** Remove fat, fibrous and necrotic areas from the tumor sample.
3. Transfer the tissue pieces into the gentleMACS C Tube containing the enzyme mix.
4. 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.
5. Run the gentleMACS Program **h_tumor_01**.
6. After termination of the program, detach C Tube from the gentleMACS Dissociator.
7. Incubate sample for 30 minutes at 37°C under continuous rotation using the MACSmix Tube Rotator.
8. 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.
9. Run the gentleMACS Program **h_tumor_01**.
10. After termination of the program, detach C Tube from the gentleMACS Dissociator.

All protocols and data sheets are available at www.miltenyibiotec.com.

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