

Contents

1. Description
 - 1.1 Principle of the Tumor Dissociation Kit
 - 1.2 Background information
 - 1.3 Applications
 - 1.4 Reagent and instrument requirements
2. Protocol
 - 2.1 Reagent preparation
 - 2.2 Tumor dissociation protocol

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 50 digestions.

The specified number of digestions is valid when digesting a tumor in a range of 0.04–1.5 g following the protocol in chapter 2.2.

Storage Store all components at 2–8 °C upon arrival. The expiration date is 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

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 mouse tumor tissue. It is optimized for a high yield of tumor cells and tumor infiltrating lymphocytes (TILs), while preserving cell surface epitopes. Dissociated cells can be subsequently cultured or isolated using MACS® Technology. Furthermore, the single-cell suspension can be analyzed *in vitro* for phenotype distributions, and other functional, genetic, or proteomic studies performed.

1.3 Applications

- Dissociation of 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

- (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)
- Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.
- 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 0.04–1.5 g is dissociated in a volume of approximately 2.5 mL enzyme mix.
- ▲ Operate MACSmix Tube Rotator with continuous rotation 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. Produce aliquots of appropriate volume. Store aliquots at –20 °C. For cell culture experiments subsequent to tissue dissociation, Solution 1 should be sterile filtered prior to aliquoting. Avoid repeated freeze-thaw-cycles.
2. Prepare Solution 2 by reconstitution of the lyophilized powder in the vial with 2.7 mL RPMI 1640. The suspension is stable for 6 months at 2–8 °C. Do not freeze.
 - ▲ **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. This solution is stable for 6 months at 2–8 °C.

2.2 Tumor dissociation protocol

1. Prepare enzyme mix by adding 2.35 mL of RPMI 1640, 100 µL of Solution 1, 50 µL of Solution 2, and 12.5 µL of Solution 3 into a gentleMACS C Tube.
2. Remove the tumor from the mouse and cut it into small pieces of 2–4 mm.
3. Transfer the tissue 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 **m_impTumor_02**.
6. After termination of the program, detach C Tube from the gentleMACS Dissociator.
7. Incubate sample for 40 minutes at 37°C with 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 **m_impTumor_03**.
 - ▲ **Note:** If strong tissue is used (e.g. 4T1 derived tumors), then run this program twice.

10. 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.
11. Resuspend sample and apply the cell suspension to a Pre-Separation Filter, 70 µm, placed on a 15 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.
12. Wash cell strainer with 10 mL of RPMI 1640.
13. Centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant completely.
14. Resuspend cells with an appropriate buffer to the required volume for further applications.
15. (Optional) To remove erythrocytes or dead cells, use Red Blood Cell Lysis Solution (10×) (# 130-094-183), or perform a density gradient centrifugation step.

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

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

autoMACS and MACS are registered trademarks and gentleMACS and MACSmix are trademarks of Miltenyi Biotec GmbH.

ART is a registered trademark and REACH is a trademark of Molecular BioProducts, Inc.

Copyright © 2012 Miltenyi Biotec GmbH. All rights reserved.