Miltenyi Biotec

Umbilical Cord Dissociation Kit
human

Order no. 130-105-737

Contents

1. Description

This product is for research use only.

Components	6 vials, containing **1 vial of Enzyme D (lyophilized powder)** **1 vial of Enzyme B (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)** **1 vial of 2.5 mL Enzyme P** **2 vials of 30 mL Buffer L**
Size	For 25 digestions. The specified number of digestions is valid when digesting umbilical cord pieces that do not exceed 0.5 g in weight following the protocols in chapter 2.2.
Storage	Upon arrival immediately store Enzyme P in aliquots at –20 °C. Store all other components at 2–8 °C upon arrival. 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 Umbilical Cord Dissociation Kit

Human umbilical cord can be dissociated to single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular adhesion proteins which maintain the structural integrity of tissues.
In a first step, the umbilical cord is soaked with enzymes which degrade the extracellular matrix. In a second step, single-cells are freed from the extracellular matrix by using the gentleMACS™ Dissociators. After dissociation remaining particles are removed by filtration using MACS® SmartStrainer.
Cells should be processed immediately for downstream applications, such as cell culture, cell separation, or cell analysis.

1.2 Background information

The Umbilical Cord Dissociation Kit, human enables the gentle and efficient generation of single-cell suspensions from human umbilical cord. The kit is not certified as GMP product and has been particularly developed to enable a quality control of frozen umbilical cord samples for umbilical cord tissue banks by detecting mesenchymal stem cells (MSCs) directly after dissociation by flow cytometry without long-standing cultivation of the cells. Additionally, the cells can be also used in downstream applications such as cell culture or molecular studies.

1.3 Applications

- Dissociation of fresh or frozen human umbilical cord pieces for subsequent cell separations using MACS Technology.
- Cultivation of umbilical cord cell populations, e.g., mesenchymal stem cells or endothelial cells.
- Phenotyping or enumeration of umbilical cord cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- PBS: phosphate-buffered saline pH 7.2
- DMEM (# 130-091-437)
- 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) 70 % ethanol
- (Optional) MACS Tissue Storage Solution (# 130-100-008)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.
- (Optional for expansion of MSCs) StemMACS™ MSC Expansion Media Kit XF, human (# 130-104-182) or StemMACS MSC Expansion Media, human (# 130-091-680)
- (Optional for differentiation of MSCs) StemMACS AdipoDiff Media, human (# 130-091-677), StemMACS ChondroDiff Media, human (# 130-091-679), or StemMACS OsteoDiff Media, human (# 130-091-678)

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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.

2.1 Reagent preparation

1. Prepare aliquots of appropriate volume of Enzyme P to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months.
2. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL Buffer L supplied with the kit. 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.
3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL Buffer L supplied with the kit. 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.
4. Prepare Enzyme B by reconstitution of the lyophilized powder in the vial with 1 mL Buffer L supplied with the kit. 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. For cell culture experiments subsequent to tissue dissociation, Enzyme B should be sterile filtered prior to aliquoting.

2.2 Umbilical cord dissociation protocol

2.2.1 Dissociation of frozen tissue

1. Cryopreservation:
   Wash umbilical cord in an appropriate buffer. Cut umbilical cord into small pieces (<5 mm diameter) to allow quick penetration of cryopreservation buffer. Add a cryoconservation medium containing 10% DMSO and transfer sample in a cryopreservation tube or bag. Freeze sample slowly at –70 °C, e.g., by using an appropriate styrofoam box. After 24 hours transfer sample into a liquid nitrogen tank.
2. Thawing of samples:
   Switch on a water bath at 37 °C. Thaw the sample (about 0.5 g of tissue) completely using the water bath. Transfer the sample into a tube containing 45 mL of DMEM. Invert the tube for 1 minute and transfer the sample onto a MACS SmartStrainer (100 µm).
3. Add 2.2 mL of Buffer L into the gentleMACS C Tube.
4. Transfer the retained sample from the strainer into the C Tube.
5. Add 100 µL of Enzyme D, 62.5 µL of Enzyme P, 4 µL of Enzyme B, and 10 µL of Enzyme A into the C Tube.

   ▲ **Note:** Enzyme A, B, and D can be premixed before addition into the C Tube. Do not premix any of the enzymes with Enzyme P.
6. Tightly close C Tube beyond the first resistance.
   If using the heating function of the gentleMACS Octo Dissociator with Heaters, attach the C Tube upside down onto the sleeves of the gentleMACS Octo Dissociator with Heaters. It has to be ensured that the sample material is still located in the area of the rotor/stator before starting the gentleMACS program. Run the gentleMACS Program **37C_h_UCDK_1** and continue with step 10.
7. Incubate sample in a water bath at 37 °C for 3 hours.
8. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.

   ▲ **Note:** It has to be ensured that the sample material is still located in the area of the rotor/stator before starting the gentleMACS program.
9. Run the gentleMACS program **h_cord_01**.
10. After termination of the program, detach C Tube from the gentleMACS Dissociator.
11. Perform a short centrifugation step to collect the sample material at the tube bottom.
12. Apply sample 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 of DMEM.

    ▲ **Note:** For maximum cell recovery, rinse the used C Tube with wash buffer before transfer to the filter.
14. Discard the filter, invert tube after closing and centrifuge sample at 300×g for 15 minutes at room temperature.
15. Aspirate supernatant completely and resuspend cells with medium or an appropriate buffer to the required volume for further applications.
16. Process cells immediately for further applications.

    ▲ **Note:** MSCs, endothelial cells, and leukocytes can be distinguished after dissociation by the surface markers CD90, CD73, CD34, and CD45 (MSCs are $CD90^+CD73^+CD34^-CD45^-$; endothelial cells are $CD34^+CD73^+CD45^-CD90^-$). If using the MSC Phenotyping Kit, be aware that CD105 is degraded during the dissociation step and that endothelial cells cannot be distinguished from leukocytes by this kit.

    ▲ **Note:** For expansion of MSCs we recommend using the StemMACS MSC Expansion Media Kit XF, human (# 130-104-182).

2.2.2 Dissociation of fresh tissue

1. Wash umbilical cord in an appropriate buffer or cell culture medium, e.g., MACS Tissue Storage Solution or DMEM.

   ▲ **Note:** For subsequent cell culture soak umbilical cord in 70 % ethanol for 30 seconds, then cut off the ends, and wash at least once in an appropriate buffer. Remove as much blood as possible.
2. Add Buffer L (volume according to the table in step 5) into the gentleMACS C Tube.
3. Cut off a piece of the umbilical cord (weight according to the table in step 5) and transfer it into the C Tube.
4. Cut the tissue in small pieces of 2–4 mm using scissors which reach the bottom of the C Tube.

5. Add enzymes into the C Tube according to the following table:

Tissue [g]	Buffer L [mL]	Enzyme D [µL]	Enzyme P [µL]	Enzyme B [µL]	Enzyme A [µL]
0.5	2.2	100	62.5	4	10
1	4.4	200	125	8	20
2–4	8.8	400	250	16	40

▲ **Note:** Enzyme A, B, and D can be premixed before addition into the C Tube. Do not premix any of the enzymes with Enzyme P.

6. Tightly close C Tube beyond the first resistance.
If using the heating function of the gentleMACS Octo Dissociator with Heaters, attach the C Tube upside down onto the sleeves of the gentleMACS Octo Dissociator with Heaters. Run the gentleMACS Program **37C_h_UCDK_1** and continue with step 10.
7. Incubate sample in a water bath at 37 °C for 3 hours.
8. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.
▲ **Note:** It has to be ensured that the sample material is still located in the area of the rotor/stator before starting the gentleMACS program.
9. Run the gentleMACS program **h_cord_01**.
10. After termination of the program, detach C Tube from the gentleMACS Dissociator.
11. Perform a short centrifugation step to collect the sample material at the tube bottom.
12. Apply sample 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 DMEM. Use twice as much DMEM as volume in tube.
▲ **Note:** For maximum cell recovery, rinse the used C Tube with wash buffer before transfer to the filter.
14. Discard the filter, invert tube after closing and centrifuge sample at 300×g for 15 minutes at room temperature.
15. Aspirate supernatant completely and resuspend cells with medium or an appropriate buffer to the required volume for further applications.
16. Process cells immediately for further applications.
▲ **Note:** MSCs, endothelial cells, and leukocytes can be distinguished after dissociation by the surface markers CD90, CD73, CD34, and CD45 (MSCs are $CD90^+CD73^+CD34^-CD45^-$; endothelial cells are $CD34^+CD73^+CD45^-CD90^-$). If using the MSC Phenotyping Kit, be aware that CD105 is degraded during the dissociation step and that endothelial cells cannot be distinguished from leukocytes by this kit.
▲ **Note:** For expansion of MSCs we recommend using the StemMACS MSC Expansion Media Kit XF, human (# 130-104-182).

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

Warranty

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Miltenyi Biotec

Whole Skin Dissociation Kit
human

Order no. 130-101-540

Contents

1. Description

Components	4 vials, containing: **2.5 mL of Enzyme P** **1 vial Enzyme A (lyophilized powder)** **1 vial Enzyme D (lyophilized powder)** **30 mL of Buffer L**
Size	For 50 digestions. The specified number of digestions is valid for 4 mm biopsies following the protocol in chapter 2.2.
Storage	Uppon arrival immediately store Enzyme P aliquoted at –20 °C. Store all other components at 2–8 °C upon arrival. The expiration date is 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 Whole Skin Dissociation Kit

Human whole skin can be dissociated into single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular adhesion proteins, which maintain the structural integrity of tissues.
Dermis is not separated from epidermis. In a first step, the human skin is soaked with enzymes which degrade the extracellular matrix. In a second step, single-cells are freed from the extracellular matrix by using the gentleMACS™ Dissociator.
Cells should be processed immediately for downstream applications, such as cell separation, cell culture, cellular or molecular analyses.

1.2 Background information

The Whole Skin Dissociation Kit, human enables the gentle and efficient generation of single-cell suspensions from human skin tissue. The kit has been particularly developed for the isolation of fibroblasts from diverse human skin biopsies. Furthermore, dissociated cells can be subsequently cultured or isolated using MACS® Technology.

1.3 Applications

- Dissociation of human skin tissue for the cultivation of fibroblasts.
- Phenotyping or enumeration of human skin cell populations by flow cytometry.

1.4 Reagent and instrument requirements

- Cell culture medium, e.g., DMEM with stable glutamine (# 130-091-438) with 10% fetal bovine serum (FBS), 10 mM HEPES, 1% Penicillin/Streptavidin, 1mM sodium pyruvat, and 1× non-essential amino acids.
- Pre-Separation Filters, 70 µm (# 130-095-823)
- gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) MACS Tissue Storage Solution (# 130-100-008)
- (Optional) Tool for taking punch biopsies (e.g., Biopsy Punch)
- (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.

▲ The protocol has been optimized for the digestion of adult human skin from breast or abdominal reduction surgery.

▲ Up to three punch biopsies (4 mm each) can be used per digestion. When working with bigger punch biopsies, cut the biopsy into pieces with a maximum diameter of 4 mm.

2.1 Reagent preparation

1. Prepare aliquots of appropriate volume of Enzyme P to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C.
2. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL Buffer L supplied with the kit. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C.

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3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL Buffer L supplied with the kit. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C.

2.2 Whole skin dissociation protocol

1. Wash human skin tissue sample in an appropriate buffer or cell culture medium, e.g., MACS Tissue Storage Solution.
2. Carefully scrape off the subcutaneous fat using a scalpel. If the diameter of the skin sample exceeds 4 mm in diameter, take one or more 4 mm diameter punches by rotating down the tool (e.g., Biopsy Punch) through epidermis and dermis. Store punches in an appropriate buffer or cell culture medium, e.g., MACS Tissue Storage Solution, until needed.
3. Transfer 435 µL of Buffer L and 12.5 µL of Enzyme P into the gentleMACS C Tube and mix carefully.

   ▲ **Note:** Some epitopes (e.g. CD4 and CD8) are sensitive for Enzyme P. If these epitopes are to be remained in the single-cell suspension, omit the addition of Enzyme P which lowers cell yields (refer to table in chapter 3.).
4. Add 50 µL of Enzyme D and 2.5 µL of Enzyme A into the C Tube and mix carefully (keep buffer at the bottom of the tube).

   ▲ **Note:** Enzyme A and Enzyme D can be premixed before addition into the C Tube. Do not premix Enzyme P with Enzyme A or Enzyme D.
5. Transfer one sample of skin tissue (4 mm) into the C Tube containing the enzyme mix and tightly close it.

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

   ▲ **Note:** Up to 3 samples (4 mm each) can be processed per C Tube if an overnight incubation is chosen in step 6.
6. Incubate sample in a water bath at 37 °C for 3 hours or overnight.

   ▲ **Note:** Longer incubation time increases cell yield.
7. After incubation dilute the sample by adding 0.5 mL of cold cell culture medium.
8. 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.
9. Run the gentleMACS Program **h_skin_01**.

   ▲ **Note:** The extracellular matrix is not completely dissociated after the dissociation step but do not repeat the step as it reduces cell yields.
10. After termination of the program, detach C Tube from the gentleMACS Dissociator.
11. Perform a short centrifugation step to collect the sample material at the tube bottom.
12. 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.
13. Wash the filter with 4 mL of cold cell culture medium.

    ▲ **Note:** (Optional) To collect remaining cells in the C Tube add the cold medium first to the C Tube and then on top of the filter.
14. Discard the filter and centrifuge cell suspension at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.
15. Resuspend cells with medium or an appropriate buffer to the required volume for further applications, for example, resuspend cells in PEB buffer for magnetic cell separation or flow cytometry.
16. Process cells immediately for further applications.

3. Appendix

Target cell yield per sample

The following table shows the dependence of target cell yield per 4 mm punch biopsy of adult abdominal skin based on incubation length and addition of Enzyme P. The yields vary depending on donor and skin locus.

	3 hours incubation	Overnight incubation
with Enzyme P	120,000 total cells 40,000 $CD90^+$ 10,000 $CD3^+$ 4,000 $CD1c^+$	380,000 total cells 80,000 $CD90^+$ 20,000 $CD3^+$ 6,000 $CD1c^+$
without Enzyme P	45,000 total cells 10,000 $CD90^+$ 3,500 $CD3^+$ 1,500 $CD1c^+$	110,000 total cells 22,000 $CD90^+$ 8,000 $CD3^+$ 3,000 $CD1c^+$

Typical staining

Epitopes which are intact after the dissociation procedure are, for example, CD90 (fibroblasts), CD45 (leukocytes), CD3 (T cells), and CD1c (dendritic cells).
Epitopes which are degraded after the dissociation procedure are, for example, CD4 and CD8. The degradation can be avoided by omitting Enzyme P but yields will be decreased.

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.

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Miltenyi Biotec

Homogenization of tissue for mRNA isolation

Contents

1. Description

1.1 Background information

The isolation of subcellular material such as mRNA from tissues or cells requires fast and thorough homogenization of the respective starting material. The gentleMACS™ Dissociators provide optimized programs that meet these requirements. In combination with M Tubes, the gentleMACS Dissociators allow the automated homogenization of tissues in a closed system, enabling sterile sample handling. A single sample or two samples can be processed in parallel.

1.2 Reagent and instrument requirements

- gentleMACS Dissociator (# 130-093-235)
- gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS M Tubes (# 130-093-236, # 130-096-335)
- µMACS™ mRNA Isolation Kits: µMACS mRNA Isolation Kit-Small Scale (# 130-075-201), µMACS mRNA Isolation Kit-Small Scale (# 130-090-276), µMACS mRNA Starting Kit (# 130-075-202), µMACS mRNA Isolation Kit-Large Scale (# 130-075-101), µMACS mRNA Isolation Kit-Large Scale (# 130-090-277)
- (Optional) Antifoam Y-30 emulsion (e.g. Sigma-Aldrich, A6457)

2. Protocol for homogenization of tissue for mRNA isolation

▲ The protocol has been tested successfully for a range of mouse tissues, such as liver, lung, brain, spleen, kidney, or heart.

▲ If working with fibrous and/or RNase-rich material, such as mouse tail, ear, skin, muscle, or pancreas it is required to prepare total RNA prior to mRNA isolation. Please refer to the gentleMACS Protocol "Homogenization of tissue for total RNA isolation".

▲ **Note:** Very hard material such as bone should not be processed since it may damage the M Tubes.

▲ Sample volumes of 1 mL or 5 mL are processed per M Tube. For details, please refer to µMACS mRNA Isolation Kit data sheets.

▲ Its molecular characteristics make RNA chemically unstable and inherently susceptible to ubiquitous RNases. It is therefore recommended to rapidly lyse samples in Lysis/Binding buffer without interruptions to minimize mRNA degradation. Avoid thawing of frozen samples before lysis.

▲ For details on the use of the gentleMACS Dissociators, refer to the gentleMACS Dissociator user manuals.

1. Choose one of the following gentleMACS Programs:
   For fresh tissue: gentleMACS Program **RNA_01**
   For frozen tissue: gentleMACS Program **RNA_02**
2. Adjust Lysis/Binding Buffer of µMACS mRNA isolation kit to room temperature.
3. Add Antifoam A to a final concentration of 0.5% to the Lysis/Binding Buffer to prevent excessive foam formation during sample homogenization.
4. Pipette Lysis/Binding Buffer with 0.5 % Antifoam into the M Tube: 1 mL (small scale kit) or 5 mL (large scale kit). For details refer to the µMACS mRNA isolation kit data sheets.
5. Transfer tissue sample into the Lysis/Binding Buffer in the M Tube.
   ▲ **Note:** Place sample directly into the buffer to avoid adherence of the tissue to the tube wall.
6. Tightly close M Tube and turn the tube upside down in one quick move ensuring that the sample material reaches the area of the rotor/stator.
7. Attach M Tube upside down onto the sleeve of the gentleMACS Dissociator.
8. Run one of the following gentleMACS Programs:
   For fresh tissue: gentleMACS Program **RNA_01**
   For frozen tissue: gentleMACS Program **RNA_02**
9. After termination of the program, detach M Tube from the gentleMACS Dissociator.
10. Centrifuge sample at 2000×g for one minute.
11. Remove the homogenized sample from the tube.
    ▲ **Note:** Homogenized tissue can be removed from the closed M Tube by pipetting through the septum-sealed opening in the center of the cap of the M Tube. Use ART 1000 REACH 1000 µL pipette tips.
12. Proceed with mRNA isolation as described in the µMACS mRNA Isolation Kit data sheets.

All gentleMACS Protocols are available at www.miltenyibiotec.com.

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Homogenization of tissue for protein extraction

Contents

1. Description

1.1 Background information

The isolation of subcellular material such as proteins from tissues or cells requires fast and thorough homogenization of the respective starting material. The gentleMACS™ Dissociators provide optimized programs that meet these requirements. In combination with M Tubes, the gentleMACS Dissociators allow the automated homogenization of tissues in a closed system.

This protocol has been developed for the homogenization of tissues to extract cytoplasmic proteins for subsequent analysis such as Western blotting.

1.2 Reagent and instrument requirements

- gentleMACS Dissociator (# 130-093-235)
- gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS M Tubes (# 130-093-236, # 130-096-335)
- Appropriate lysis buffer for the protein of interest
- (Optional) Antifoam Y-30 emulsion (e.g. Sigma-Aldrich®, # A6457)

2. Protocol for homogenization of tissue for protein extraction

▲ The protocol has been tested successfully for a range of mouse tissues, such as liver, kidney, spleen, heart, lung, brain, skin, muscle, or intestine.

▲ **Note:** Very hard material such as bone, cartilage, or mouse tail should not be processed since it may damage the M Tubes.

▲ The sample volume should be between 300 µL and a maximum of 10 mL of lysis buffer. It is recommended to use up to 10 mg tissue per mL of lysis buffer.

▲ Pre-cool lysis buffer on ice.

▲ For details on the use of the gentleMACS Dissociators, refer to the gentleMACS Dissociator user manuals.

1. Choose the gentleMACS Program **Protein_01.**
2. Pipette appropriate amount of ice-cold lysis buffer into the M Tube.
3. (Optional) To avoid excessive foam formation during sample homogenization, add Antifoam Y-30 to a final concentration of 1% to the lysis buffer.
4. Transfer tissue sample into the lysis buffer in the M Tube.

   ▲ **Note:** Place sample directly into the buffer to avoid adherence of the tissue to the tube wall.
5. Tightly close M Tube and turn the tube upside down in one quick move ensuring that the sample material reaches the area of the rotor/stator.
6. Attach M Tube upside down onto the sleeve of the gentleMACS Dissociator.
7. Run the gentleMACS Program **Protein_01**.
8. After termination of the program, detach M Tube from the gentleMACS Dissociator.
9. Centrifuge sample at 4000×g for 5 minutes to collect lysate at the tube bottom.
10. Store on ice until proceeding with protein analysis.

All gentleMACS Protocols are available at **www.miltenyibiotec.com**.

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Homogenization of tissue for total RNA isolation

Contents

1. Description

1.1 Background information

The isolation of subcellular material such as total RNA from tissues or cells requires fast and thorough homogenization of the respective starting material. The gentleMACS™ Dissociators provide optimized programs that meet these requirements. In combination with M Tubes, the gentleMACS Dissociators allow the automated homogenization of tissues in a closed system, enabling sterile sample handling. A single sample or two samples can be processed in parallel.

1.2 Reagent and instrument requirements

- gentleMACS Dissociator (# 130-093-235)
- gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS M Tubes (# 130-093-236, # 130-096-335)
- Total RNA isolation kits from different suppliers

2. Protocol for homogenization of tissue for total RNA isolation

▲ The protocol has been tested successfully for a broad range of tissues such as liver, lung, brain, spleen, kidney, muscle, hypothalamus, intestine, bladder, heart, or skin.

▲ **Note:** Very hard material such as bone should not be processed since it may damage the M Tubes.

▲ The sample volume should be between min. 350 µL and max. 10 mL of lysis buffer.

▲ Its molecular characteristics make RNA chemically unstable and inherently susceptible to ubiquitous RNases. It is therefore recommended to rapidly lyse samples in Lysis/Binding buffer without interruptions to minimize RNA degradation. Avoid thawing of frozen samples before lysis.

▲ For details on the use of the gentleMACS Dissociators, refer to the gentleMACS Dissociator user manuals.

1. Choose one of the following gentleMACS Programs:
   For fresh tissue: gentleMACS Program **RNA_01**
   For frozen tissue: gentleMACS Program **RNA_02**
2. Adjust lysis buffer to room temperature.
3. According to the kit manufacturer's recommendations pipette an appropriate amount of lysis buffer provided by the total RNA isolation kit into the M Tube.
4. Transfer tissue sample into the Lysis Buffer in the M Tube.
   ▲ **Note:** Place sample directly into the buffer to avoid adherence of the tissue to the tube wall.
5. Tightly close M Tube and turn the tube upside down in one quick move ensuring that the sample material reaches the area of the rotor/stator.
6. Attach it upside down onto the sleeve of the gentleMACS Dissociator.
7. Run one of the following gentleMACS Programs:
   For fresh tissue: gentleMACS Program **RNA_01**
   For frozen tissue: gentleMACS Program **RNA_02**
8. After termination of the program, detach M Tube from the gentleMACS Dissociator.
9. (Optional) For sample volumes below 3 mL or if excessive foam formation occurred during the homogenization process, centrifuge M Tube at 2000×g for 1 minute to collect lysate at the tube bottom.
10. Remove the homogenized sample from the tube.
    ▲ **Note:** Homogenized tissue can be removed from the closed M Tube by pipetting through the septum-sealed opening in the center of the cap of the M Tube. Use ART 1000 REACH 1000 µL pipette tips.
11. Proceed with total RNA isolation according to the kit manufacturer's recommendations.

All gentleMACS Protocols are available at www.miltenyibiotec.com.

140-002-297.03

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Mitochondria Extraction Kit – Tissue

Order no. 130-097-340

Contents

1. Description

Components	**1 mL Solution 1** **50 mL 10×Solution 2** **52.5 mL Solution 3**
Capacity	For 25 extractions. The specified number of extractions is valid when processing tissue in the range of 50–100 mg following the protocol in chapter 2.1.2
Storage	Store solutions protected from light at 2–8 °C. The expiration date is indicated on the label.

1.1 Background information

Mitochondria dysfunction is involved in neurodegenerative diseases, such as Alzheimer or Parkinson, but also plays a pivotal role in diabetes, cancer, heart, kidney, or liver diseases. The Mitochondria Extraction Kit – Tissue has been developed for use with the gentleMACS™ Dissociators, allowing the time-saving and reliable homogenization of human or mouse tissues, including muscle, heart, liver, brain, or kidney. The resulting tissue homogenates provide an ideal basis for subsequent magnetic labeling and isolation of intact, functional mitochondria at high yield, using the Mitochondria Isolation Kit, human (# 130-094-532) or the Mitochondria Isolation Kit, mouse tissue (# 130-096-946).

1.2 Application

- Homogenization of human or mouse tissue for subsequent isolation of intact, functional mitochondria.

1.3 Reagent and instrument requirements

Tissue homogenization for mitochondrial extraction

- Human or mouse tissue
- Protease inhibitors (e.g., cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail Tablets provided in EASYpacks by Roche # 04 693 159 001)
- Cell culture dishes (35 mm)
- Dissection scissors and forceps
- gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- Pre-Separation Filters, 70 µm (# 130-095-823)

Additional reagents required for subsequent mitochondria isolation

- Mitochondria Isolation Kit, mouse tissue (#130-096-946), containing: Anti-TOM22 MicroBeads, mouse, 10× Separation Buffer, Storage Buffer, LS Columns, and Pre-Separation Filters, 30 µm

  or

- Mitochondria Isolation Kit, human (#130-094-532), containing: Anti-TOM22 MicroBeads, human, Lysis Buffer, 10× Separation Buffer, Storage Buffer, LS Columns
- For isolation of mitochondria from human tissue: Pre-Separation Filters, 30 µm (#130-041-407)
- Cooled table-top centrifuge
- MACSmix™ Tube Rotator (# 130-090-753)
- MACS® MultiStand (# 130-042-303)
- MidiMACS™ Separator (# 130-042-302) or QuadroMACS™ Separator (# 130-090-976)
- 15 mL propylene conical tubes
- 1.5 mL microcentrifuge tubes

140-003-568.01

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2. Protocol for the preparation of mitochondria from tissue

2.1 Tissue homogenization for mitochondria extraction

▲ All buffers should be pre-cooled on ice before use.

2.1.1 Reagent preparation

1. Prepare 2.1 mL aliquots of Solution 3 and store at –20 °C.

   ▲ **Note:** 2 mL of Solution 3 are required for the homogenization of 50-100 mg tissue. Before use, thaw aliquot and keep on ice.

2. Prepare 10 mL Protease Inhibition Buffer: Add 1 mL of 10× Solution 2 to 9 mL double-distilled water and dissolve one protease inhibitor tablet in the freshly prepared 1× Solution 2.

   ▲ **Note:** 2 mL of Protease Inhibition Buffer are required for the homogenization of 50–100 mg tissue. Aliquots of 2 mL can be stored at -20 °C for 4 weeks.

3. Prepare 1× Solution 2 by adding 1 volume of 10× Solution 2 to 9 volumes of double-distilled water (H_20 bidest.), i.e., add 1.8 mL of 10× solution 2 to 16.2 mL of H_20 bidest.

   ▲ **Note:** Up to 18 mL of 1× Solution 2 are required for the homogenization of 50–100 mg tissue. Always use freshly prepared 1× Solution 2.

4. Prepare Extraction Buffer by adding 20 µL of Solution 1 to 0.5 mL of freshly prepared 1× Solution 2.

2.1.2 Tissue processing

▲ For details on the use of the gentleMACS Dissociators, refer to the gentleMACS Dissociator user manuals.

▲ Tissue in the range of 50–100 mg is homogenized in a C Tube in a total volume of 2 mL Protease Inhibition Buffer.

▲ **Note:** Up to 500 mg tissue in a maximum total volume of 10 mL can be dissociated per gentleMACS C Tube.

1. Rinse tissue twice in 3–4 mL ice-cold 1× Solution 2.
2. Add 3 mL ice-cold 1× Solution 2 to a 35 mm cell culture dish and place the dish on ice. Transfer tissue into the cell culture dish.

   ▲ **Note:** The tissue should be covered with liquid.

3. Resect fat, ligament, and connective tissue using a forceps and a pair of dissection scissors. Cut the tissue into pieces of approximately 5 mm.
4. Determine the weight of the tissue: Fill a second 35 mm cell culture dish with 3 mL ice-cold 1× Solution 2 and use this dish as tare weight. Quickly and gently dab the tissue pieces on a paper towel before placing them in the prepared culture dish. Measure the weight of the tissue.
5. Transfer 50–100 mg tissue to a 1.5 mL microcentrifuge tube containing 0.5 mL ice-cold Extraction Buffer.
6. Quickly mince tissue into small pieces of approximately 1–2 mm using a pair of dissection scissors. Incubate for 30 minutes on ice.
7. Centrifuge at 300×g for 5 minutes at 4 °C and discard the supernatant.
8. Add 1 mL ice-cold Protease Inhibition Buffer, gently flick the tube to resuspend the pellet and pour suspension into a pre-cooled gentleMACS C Tube. Rinse the microcentrifuge tube with an additional 1 mL of ice-cold Protease Inhibition Buffer and pour the suspension into the same gentleMACS C Tube.

   ▲ **Note:** Instead of pouring the suspension, a glass Pasteur pipette can also be used. Use of a plastic pipette tip is not recommended as small tissue pieces tend to stick to the wall of the tip.

9. 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 rotator/stator.

10. Run the gentleMACS Program.

    For mouse tissue: m_mito_tissue_01.

    For human tissue: h_mito_tissue_01.

11. After termination of the program, detach C Tube from the gentleMACS Dissociator and perform a short centrifugation step at 200×g for 30 seconds at 4 °C to collect the homogenate at the tube bottom.
12. Put a 15 mL conical tube on ice and place a Pre-Separation Filter, 70 µm on top. Remove homogenate from C Tube, pipette it into the reservoir of the filter and allow the homogenate to run through.
13. Wash filter with 2×1 mL ice-cold Solution 3. Centrifuge the filtered homogenate at 500×g for 5 minutes at 4 °C. Transfer supernatant containing mitochondria to a 15 mL conical tube and immediately proceed to 2.3 Mitochondria isolation.

140-003-568.01

2.2 Protocol overview

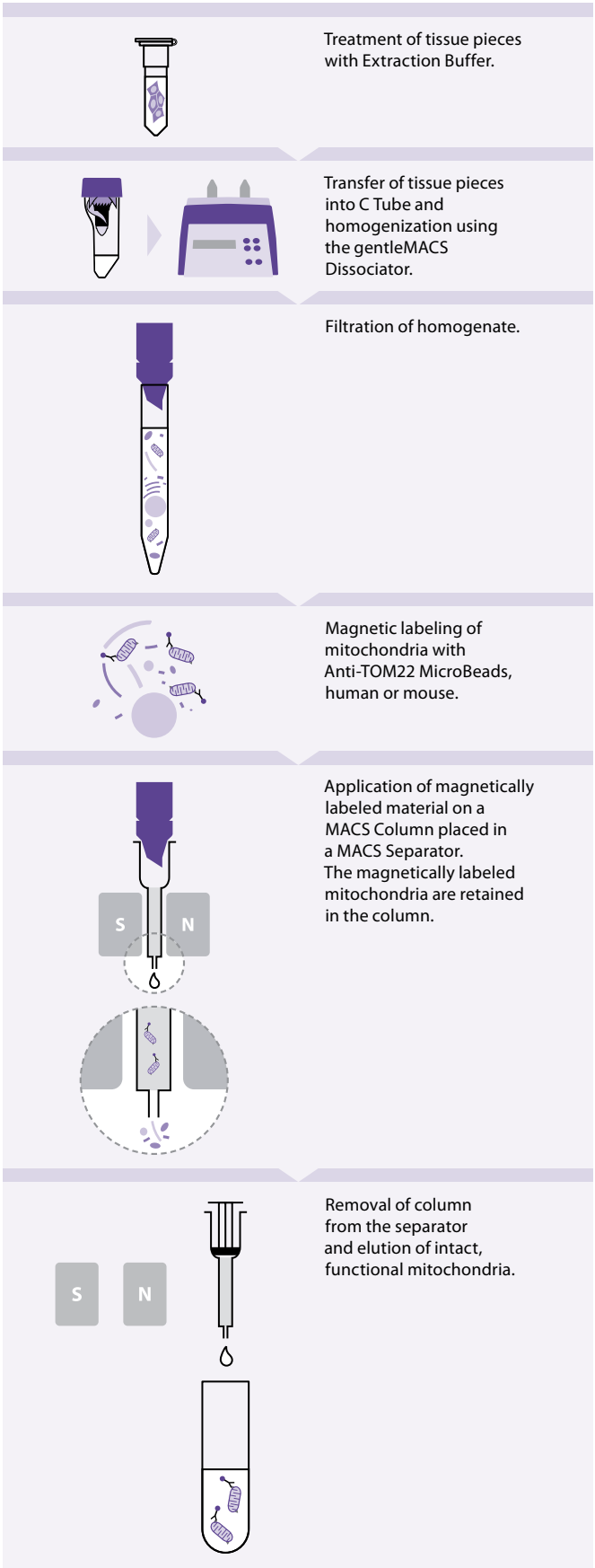

Figure 1: Isolation of mitochondria from human and mouse tissue.

2.3 Mitochondria isolation

▲ Magnetic labeling and separation is performed with homogenates derived from 50–100 mg tissue. Split homogenates derived from larger amounts of tissue into portions.

2.3.1 Reagent preparation

To prepare 23 mL of 1× Separation Buffer, incubate 10× Separation Buffer at 37 °C until visible crystals are no longer present and add 2.3 mL 10× Separation Buffer to 20.7 mL H_2O bidest. Pre-cool 1× Separation Buffer on ice.

▲ **Note:** Aliquots of 2.3 mL 10× Separation Buffer can stored at –20 °C.

2.3.2 Magnetic labeling and separation

1. Take supernatant derived from 50–100 mg tissue (section 2.1.2, step 13) and add ice-cold 1× Separation Buffer to a total volume of 10 mL. Mix well.
2. Add 50 µL Anti-TOM22 MicroBeads, mouse or 50 µL Anti-TOM22 MicroBeads, human to magnetically label the mitochondria.
3. Mix well and incubate for 1 hour in the refrigerator (2–8 °C) under continuous rotation using the MACSmix Tube Rotator.

   ▲ **Note:** Operate MACSmix Tube Rotator on permanent run at a speed of approximately 12 rpm.
4. Place LS Column into the magnetic field of a suitable MACS Separator and place a Pre-Separation Filter, 30 µm on top of the LS Column.
5. Prepare column by rinsing with 3 mL of 1× Separation Buffer.
6. Apply the magnetically labeled mitochondria onto the column and let the homogenate run through.

   ▲ **Note:** Apply homogenate in aliquots of 3×3.3 mL. Add aliquots only when the column reservoir is empty.
7. Wash column with 3×3 mL of 1× Separation Buffer.

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
8. Remove column from the separator and place it on a 15 mL conical tube.
9. Pipette 1.5 mL of 1× Separation Buffer onto the column. Immediately flush out the enriched mitochondria by firmly pushing the plunger into the column.
10. Proceed with downstream analysis.

    ▲ **Note:** The 1× Separation Buffer contains stabilizing agent. Before measurement of mitochondria protein content please carry out the following procedure:

    1. Centrifuge the isolated mitochondria at 13,000×g for 2 minutes at 4 °C. Aspirate the supernatant.
    2. Resuspend the mitochondria pellet in 1,000 µL of Storage Buffer.
    3. Centrifuge mitochondria suspension at 13,000×g for 2 minutes at 4 °C. Aspirate the supernatant.
    4. Resuspend the mitochondria pellet in 100 µL Storage Buffer.
11. (Optional) If mitochondria are not immediately used for downstream analysis centrifuge mitochondria suspension at 13,000×g for 2 minutes at 4 °C. Aspirate the supernatant. Resuspend the mitochondria pellet in 100 µL Storage Buffer supplied with the Mitochondria Isolation Kit and store on ice. Storage should not exceed two hours to preserve high quality mitochondria. For functional analysis of mitochondrial respiration, optimal results are achieved when mitochondria are used immediately.

140-003-568.01

3. Appendix

For optimal results it is recommended to homogenize tissue samples by using the gentleMACS Dissociators and the respective gentleMACS Programs. Alternatively, a dounce homogenizer for tissue dissociation can be used, as described elsewhere [1,2]. Follow the protocol in section 2.1.2, steps 1–7. Then, homogenize the tissue pieces using the dounce homogenizer by adding ice-cold Protease Inhibition Buffer at a tissue:buffer ratio of 1:10 (e.g., homogenize 100 mg tissue in 1 mL Protease Inhibition Buffer). After homogenization immediately proceed to 2.3 Mitochondria isolation.

4. References

1. Frezza, C. *et al.* (2007) Organelle isolation: functional mitochondria from mouse liver, muscle and cultured fibroblasts. Nat. Prot. 2(2): 287–295.
2. Wieckowski, M.R. *et al.* (2009) Isolation of mitochondria associated membranes and mitochondria from animal tissues and cells. Nat. Prot. 4: 1582–1590.

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

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Preparation of single-cell suspensions from mouse spleen without enzymatic treatment

Contents

1. Description

1.1 Background information

Single-cell suspensions are a prerequisite for many experiments, for example to achieve the highest possible purity and recovery during cell separations with MACS® Technology. The gentleMACS™ Dissociator provides optimized programs to attain single-cell suspensions from various tissues, for example, mouse spleen. In combination with C Tubes, the gentleMACS Dissociator allows the automated tissue dissociation in a closed system, enabling sterile sample handling. A single tube or up to eight tubes can be processed in parallel.

1.2 Reagent and instrument requirements

- 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)
- Pre-Separation Filters, 30 µm, (# 130-041-407)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.
- 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).

2. Protocol for the dissociation of mouse spleen

▲ 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 spleen amounts to 80–120 mg (female BALB/c mouse, 6–7 weeks old).

1. Transfer mouse spleen into the gentleMACS C Tube containing buffer:

   1–2 mouse spleens: 3 mL

   3–4 mouse spleens: 6 mL

   5–6 mouse spleens: 9 mL

2. 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.

3. Choose one of the gentleMACS Programs:

   1–2 mouse spleens: **m_spleen_01**.

   3–6 mouse spleens: **m_spleen_04**.

4. Run the gentleMACS Program **m_spleen_01** or **m_spleen_04**.
5. After termination of the program, detach C Tube from the gentleMACS Dissociator.
6. (Optional) Perform a short centrifugation step to collect the sample material at the bottom of the tube.
7. Resuspend sample and apply the cell suspension to a Pre-Separation Filter, 30 µm, placed on a 15 mL tube (1–2 mouse spleens per C Tube) or to an appropriate cell strainer placed on a 50 mL tube (3–6 mouse spleens per C 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.

8. Wash Pre-Separation Filter with 5 mL of buffer.
9. Discard Pre-Separation Filter and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
10. Resuspend cells with buffer to the required volume for further applications.

All gentleMACS Protocols are available at www.miltenyibiotec.com.

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Miltenyi Biotec

Adipose Tissue Dissociation Kit
mouse and rat

Order no. 130-105-808

Contents

1. Description

Components	5 vials, containing: **2 vials of Enzyme D (lyophilized powder)** **1 vial of Enzyme R (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)** **1 mL of Buffer A**
Size	For 50 digestions of 2.5 mL.
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 Adipose Tissue Dissociation Kit

Mouse or rat adipose 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 adipose tissue is enzymatically digested using the kit components and the gentleMACS™ Dissociator is 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 Adipose Tissue Dissociation Kit, mouse and rat has been developed for the gentle, rapid, and effective generation of single-cell suspensions from mouse and rat adipose tissue. It is optimized for a high yield of viable cells, 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 mouse or rat adipose tissue into single-cell suspensions for subsequent cell separations using MACS Technology.
- Cultivation of adipose tissue resident cell populations.
- Phenotyping or enumeration of cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- DMEM (# 130-091-437)
- MACS SmartStrainers, 100 µm (# 130-098-463)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C.
- 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) MACS Tissue Storage Solution (# 130-100-008)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.
- (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 mouse or rat serum albumin, mouse or rat serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

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140-004-708.01

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.

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

▲ Appropriate volume of enzyme mix based on tissue volume:

	White adipose tissue	Brown adipose tissue
Up to 0.5 g tissue	2.5 mL	1.25 mL
0.51–1.0 g tissue	5 mL	2.5 mL

If more than 1.0 g of tissue has to be digested use more tubes.

2.1 Reagent preparation

1. Prepare Enzyme D by reconstitution of the lyophilized powder in each vial with 3 mL of 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 of 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 of Buffer A supplied with the kit. 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 Adipose tissue dissociation protocol

1. Prepare enzyme mix by adding 2.35 mL of DMEM, 100 µL of Enzyme D, 50 µL of Enzyme R, and 12.5 µL of Enzyme A into a gentleMACS C Tube for a dissociation volume of 2.5 mL.
2. Resect the adipose tissue and cut it into small pieces of 2–4 mm. Refer to table in section 2 for the appropriate volume.
3. Transfer the tissue into the gentleMACS C Tube containing the enzyme mix and tightly close it.
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_mr_ATDK_1** and continue with step 11.

   ▲ **Note:** Close C Tube tightly beyond the first resistance.
4. Incubate sample for 20 minutes at 37 °C under continuous agitation using the MACSmix Tube Rotator.
5. 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.
6. Run the gentleMACS Program **mr_adipose_01**.
7. After termination of the program, detach C Tube from the gentleMACS Dissociator.
8. Incubate sample for 20 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 **mr_adipose_01**.
11. After termination of the program, detach C Tube from the gentleMACS Dissociator.
12. (Optional) Perform a short centrifugation step up to 300×g to collect the sample material at the tube bottom.
13. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (100 µm) placed on a 15 mL or 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.
14. Wash MACS SmartStrainer (100 µm) with 5–10 mL of DMEM.
15. Discard the MACS SmartStrainer (100 µm) and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
16. Resuspend cells with an appropriate buffer to the required volume for further applications, for example, resuspend cells in PEB buffer for magnetic cell separation or flow cytometry.
17. Process cells immediately for further applications.

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

Warranty

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140-004-708.01

Miltenyi Biotec

Brain Tumor Dissociation Kits

Brain Tumor Dissociation Kit (P)	130-095-942
Brain Tumor Dissociation Kit (T)	130-095-939

Contents

1. Description

This product is for research use only.

Components

Brain Tumor Dissociation Kit (P)
6 vials, containing:
1.25 mL of Enzyme N
2×50 mL of Buffer X (sterile)
1.5 mL of Buffer Y (sterile)
1 vial of Enzyme A
1 mL of Buffer A

or

Brain Tumor Dissociation Kit (T)
6 vials, containing:
4 mL of Enzyme U
2×50 mL of Buffer X (sterile)
1.5 mL of Buffer Y (sterile)
1 vial of Enzyme A
1 mL of Buffer A

Size
For 25 digestions of 4 mL.

Storage
Upon arrival immediately store Enzyme U of the Brain Tumor Dissociation Kit (T) aliquoted at –20 °C.
Store all other components at 2–8 °C upon arrival. The expiration date is indicated on the box label. For information about reconstitution and storage after reconstitution of the lyophilized component refer to chapter 2.1.

1.1 Principle of the Brain Tumor Dissociation Kits

Human brain tumors can be dissociated to single-cell suspensions by enzymatic digestion of the extracellular adhesion proteins.
The human brain tumor, for example, primary glioblastoma, can be dissociated either using the gentleMACS™ Dissociators or manually using Pasteur pipettes. The tissue is cut into small pieces, then digested enzymatically, and further mechanically dissociated into a single-cell suspension by trituration. Optionally, myelin can be removed using Myelin Removal Beads II, as it can interfere with subsequent flow cytometric analysis or cell separation using MACS® Technology.

1.2 Background information

The Brain Tumor Dissociation Kits (BTDK) and the corresponding gentleMACS Programs have been designed for a gentle but rapid and efficient generation of single-cell suspensions from human brain tumors. This reliable standardized protocol is a prequisite for successful downstream applications such as magnetic cell sorting. The isolation and characterization of specific cell populations within a tumor, e.g., cancer stem cells, is important for the analysis of cancerous potential and the development of therapies.

1.3 Applications

- Dissociation of human brain tumors to single-cell suspensions for subsequent cell separations using MACS Technology.
- Dissociation of human brain tumors to single-cell suspensions for subsequent *in vitro* cultivation, e.g. neurosphere assay.
- Enumeration and phenotyping of individual human tumor cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- Hanks' Balanced Salt Solution (HBSS) without Ca^{2+} and Mg^{2+} (Sigma-Aldrich # 55021C), in the following referred to as HBSS (w/o)
- HBSS with Ca^{2+} and Mg^{2+} (Sigma-Aldrich # 55037C), in the following referred to as HBSS (w)
- (Optional) Beta-mercaptoethanol, 50mM
- 15 mL and 50 mL tubes
- MACS SmartStrainer (70 µm) (# 130-098-462) for 50 mL tubes
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubation oven at 37 °C
- (Optional) gentleMACS Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427) and 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.
- (Optional) Myelin Removal Beads II, human, mouse, rat (# 130-096-733, # 130-096-433)

Additional for manual dissociation:

- (Sterile) scalpel
- 35 mm diameter sterile petri dish
- (Sterile) glass Pasteur pipettes

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140-003-192.06

2. Protocol

2.1 Reagent and instrument preparation

▲ For optional dissociation of neural tissue in combination with the gentleMACS™ Dissociators, please refer to section 2.2.1. For manual dissociation of neural tissue refer to section 2.2.2.

▲ Volumes given below are for up to 800 mg of starting tissue material. When working with less than 800 mg, use the same volumes as indicated. Tissue quantities of greater than 800 mg can be pooled and processed in an appropriate-sized conical tube. When working with more than 800 mg, scale up all reagent volumes and total volumes accordingly.

1. (Optional for increased stability of enzymes) Add beta-mercaptoethanol to Buffer X to a final concentration of 0.067 mM. For example, add 13.5 µL of 50 mM beta-mercaptoethanol to 10 mL of Buffer X.

   ▲ **Note:** This solution will then be stable for 1 month at 4 °C.

2. Resuspend the lyophilized powder in the vial labeled Enzyme A with 1 mL Buffer A. Do not vortex. This solution should then be aliquoted and stored at –20 °C for later use.

	Volumes needed for up to 800 mg of tissue			
BTDK (P)	Enzyme N 50 µL	Buffer X 3890 µL	Buffer Y 40 µL	Enzyme A 20 µL
BTDK (T)	Enzyme U 160 µL	Buffer X 3780 µL	Buffer Y 40 µL	Enzyme A 20 µL

2.2 Brain tumor dissociation protocol

▲ The tumor sample should be stored in phosphate-buffered saline (PBS) until processing.

▲ Remove necrotic tissue from the tumor sample.

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ In case of subsequent gene expression profiling perform all steps at 4 °C instead of room temperature.

▲ This protocol describes the dissociation of human primary glioblastoma, though, in principle, it is transferable to other brain tumor tissue types.

▲ The MACSmix™ Tube Rotator is used with continuous ratation at a speed of approximately 4 rpm.

2.2.1 Automated dissociation using the gentleMACS™ Dissociators

▲ For details on the use of the gentleMACS™ Dissociators, refer to the gentleMACS Dissociator user manuals.

▲ A maximum of 1600 mg human brain per C Tube can be processed. The total volume should not exceed 10 mL, minimum volume is 4 mL.

1. Determine the weight of tissue after discarding the buffer (PBS).
2. Transfer the appropriate volume of Buffer X (refer to table in section 2.1) into a gentleMACS C Tube and pre-heat at 37 °C for 10–15 minutes before use.

   ▲ **Note:** Preheating is not required if using the heating function of the gentleMACS Octo Dissociator with Heaters.

3. Transfer the tissue into the C Tube containing the pre-heated Buffer X.

   ▲ **Note:** If very strong tissue is used, then cut it first into smaller pieces using a scalpel.

4. Add the appropriate volume of Enzyme N or U, Buffer Y, and Enzyme A (refer to table in section 2.1) to the C Tube and mix gently.
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 to the upper right of the rotor blade.

6. Run the gentleMACS Program **h_tumor_02**. If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_BTDK_1** and continue with step 13.
7. After termination of the program, detach C Tube from the gentleMACS Dissociator.
8. Incubate sample for 15 minutes at 37 °C under slow, continuous rotation using the MACSmix Tube Rotator.
9. Run the gentleMACS Program **h_tumor_03**.
10. After termination of the program, detach C Tube from the gentleMACS Dissociator.
11. Incubate sample for 10 minutes at 37 °C under slow, continuous rotation using the MACSmix Tube Rotator.
12. Run gentleMACS Program **m_brain_01**.
13. After termination of the program, detach C Tube from the gentleMACS Dissociator.
14. Centrifuge briefly to collect the sample at the bottom of the tube.
15. Resuspend sample and apply it to a MACS® SmartStrainer (70 µm) placed on a 50 mL tube.

    ▲ **Note:** When upscaling the reagent volume and total volumes, increase also the number of MACS SmartStrainers (70 µm). One MACS SmartStrainer (70 µm) can be used for up to 4 mL.

    ▲ **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.

    ▲ **Note:** Cells with a diameter >70 µm may be lost. To obtain these cells within the flow through, use a cell strainer with an appropriate mesh size.

16. Apply 20 mL of HBSS (w) through MACS SmartStrainer (70 µm).

    ▲ **Note:** When working with more than 800 mg human brain wash MACS SmartStrainer (70 µm) with an appropriate amount of HBSS (w), five times the enzyme solution volume. If necessary split the sample.

17. Discard MACS SmartStrainer (70 µm) and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
18. (Optional) Resuspend cell suspension in 20 mL HBSS (w) and centrifuge at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
19. Resuspend cells with buffer to the required volume for further applications.
20. (Optional) If myelin is present it is recommend to use Myelin Removal Beads II. For details refer to the Myelin Removal Beads II data sheet.
21. Cells should be processed immediately for further applications.

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2.2.2 Manual dissociation

1. Fire-polish three glass Pasteur pipettes so that decreasing tip diameters are achieved. For details refer to 3. Appendix.
2. Pre-heat the appropriate volume of Buffer X (refer to table in section 2.1) at 37 °C for 10–15 minutes before use.
3. Determine the weight of tissue by discarding the buffer (PBS). Place the tissue on a petri dish.
4. Cut the tumor into small pieces using a scalpel.
5. Using a 1 mL pipette tip, add 1 mL of HBSS (w/o) and pipette pieces back into a 15 mL tube. Rinse with HBSS (w/o).
6. Centrifuge at 300×g for 2 minutes at room temperature and aspirate the supernatant carefully.
7. Add 4 mL of pre-heated Buffer X per 800 mg tissue.
8. Add the appropriate volume of Enzyme N or U, Buffer Y, and Enzyme A (refer to table in section 2.1) to the tube and mix gently.
9. Incubate the closed tube for 15 minutes at 37 °C under slow, continuous rotation using a MACSmix™ Tube Rotator.
10. Dissociate tissue mechanically using the wide-tipped, fire-polished Pasteur pipette by pipetting up and down 10 times slowly. Avoid forming air bubbles.

    ▲ **Note:** If the pipette is blocked by tissue pieces, repeat this step once or twice.
11. Incubate at 37 °C for 10 minutes using the MACSmix™ Tube Rotator.
12. Dissociate tissue mechanically using the other two fire-polished Pasteur pipettes in decreasing diameter. Pipette slowly up and down 10 times with each pipette, or as long as tissue pieces are still observable. Be careful to avoid the formation of air bubbles.
13. Apply the cell suspension to a MACS® SmartStrainer (70 µm) placed on a 50 mL tube.

    ▲ **Note:** When upscaling the reagent volume and total volumes, increase also the number of MACS SmartStrainers (70 µm). One MACS SmartStrainer (70 µm) can be used for up to 4 mL.

    ▲ **Note:** Cells with a diameter >70 µm may be lost. To obtain these cells within the flow-through, use a cell strainer with an appropriate mesh size.
14. Apply 20 mL of HBSS (w) through MACS SmartStrainer (70 µm).

    ▲ **Note:** When working with more than 800 mg human brain wash MACS SmartStrainer (70 µm) with an appropriate amount of HBSS (w), five times the enzyme solution volume. If necessary split the sample.
15. Discard MACS SmartStrainer (70 µm) and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
16. (Optional) Resuspend cell suspension in 20 mL HBSS (w) and centrifuge at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
17. Resuspend cells with buffer to the required volume for further applications.
18. (Optional) If myelin is present it is recommend to use Myelin Removal Beads II. For more details refer to the Myelin Removal Beads II data sheet.
19. Cells should be processed immediately for further applications.

3. Appendix: Tips & hints

▲ For up-to-date information regarding antigen compatibilities of Brain Tumor Dissociation Kits for subsequent MACS Cell Separations, please refer to www.miltenyibiotec.com.

Production of appropriate Pasteur pipettes
For the manual dissociation protocol, three Pasteur pipettes with openings of decreasing diameter are needed. The opening of the first pipette should be rounded without significant decrease in the size of the opening. The smallest opening should not be smaller than 0.5 mm so that the cells are not forced through with too much pressure. To produce openings that get progressively smaller, rotate the pipettes quickly in the flame to fire-polish them for a few seconds. Production is easier if you apply the rubber sucker. Too much time may fuse the opening. The edges should be rounded.

Yield of viable cells is too low (dissociation is insufficient)
Make sure that the tissue pieces are agitated sufficiently during the entire time of incubation and do not stick to the bottom of the tube. Flick or invert the tube after adding the enzyme mixes if it is necessary. During the working steps at 37 °C the MACSmix Tube Rotator is convenient for this purpose. Apply the suspension to a cell strainer with a pore size appropriate for the size of the target cells.

Formation of a pellet after washing is inhibited by sticky threads or particles
Add another 30 µL enzyme mix per 2 mL, incubate for 5–10 minutes at 37 °C, centrifuge, and wash again.

Single-cell suspension contains only dead cells
Make sure the openings of the Pasteur pipettes are not too small. Pipette more slowly and do not vortex the cells. Avoid forming bubbles. Follow the protocol non-stop.

Purity is low after separation using MACS® MicroBeads
If myelin is present it is recommend to use the Myelin Removal Beads II, because myelin impairs the specific binding of antibodies and therefore the separation of cells.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols.

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Miltenyi Biotec

Embryoid Body Dissociation Kit
human and mouse

Order no. 130-096-348

Contents

1. Description

Components 6 vials, containing
2.5 mL Enzyme P
2×50 mL Buffer X
1.5 mL Buffer Y
1 vial Enzyme A (lyophilized powder)
1 mL Buffer A

Size For 50 digestions.
The specified number of digestions is valid when embryoid bodies at several stages generated from a starting cell number of 1×10^6/10 mL (mouse) and 4×10^6/10 mL (human) are used.

Storage Upon arrival immediately store Enzyme P in aliquots at –20 °C. Store all other components at 2–8 °C upon arrival. Reconstitute Enzyme A before the date indicated on the box label. For information about reconstitution and storage after reconstitution of the lyophilized component refer to chapter 2.1.

1.1 Background information

Embryoid body (EB) formation is a crucial step in many ES or iPS cell differentiation protocols. For subsequent cell analysis or isolation of specific cell populations of the EB, single-cell suspensions are a prerequisite, for example, to achieve the highest possible purity and recovery during cell separations with MACS® Technology. The gentleMACS™ Dissociator provides optimized programs to attain single-cell suspensions from *in vitro* generated EBs.
EBs are cellular aggregates generated from differentiating pluripotent stem cells grown in suspension in defined cell numbers and media. Cell aggregation is initiated by suspension culture in hanging drops or as mass suspension culture in non-tissue culture plates or spinner flasks. During the three dimensional differentiation process, *in vivo* embryonic development is recapitulated to a limited extent and differentiated cell types of all three germ layers are generated. Embryoid bodies are a well studied model system for investigating early stages of development and deriving distinct cell types from pluripotent stem cells.

1.2 Applications

- Dissociation of EBs into single-cell suspensions for subsequent cell separations using MACS Technology.
- Cultivation of EB-derived cells.
- Phenotyping or enumeration of individual EB-derived cell populations by flow cytometry.
- Dissociation of pluripotent stem cell-derived neurospheres.

▲ **Note:** The Embryoid Body Dissociation Kit is not suitable for the dissociation of tissue-derived neurospheres. In this case use the Neurosphere Dissociation Kit (P) (# 130-095-943) or the Neurosphere Dissociation Kit (T) (# 130-095-944) depending on the antigen epitope of interest.

1.3 Reagent and instrument requirements

- Buffer: Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+}
- EB culture medium
- Pre-Separation Filters, 70 µm (# 130-095-823)
- (Optional) gentleMACS Dissociator (# 130-093-235)
- (Optional) gentleMACS M Tubes (# 130-093-236, # 130-096-335)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed M Tubes.

2. Protocol

▲ The dissociation protocols have been optimized for embryoid bodies generated from a starting cell number of 1×10^6/10 mL (mouse) and 4×10^6/10 mL (human).

▲ For cell culture experiments subsequent to embryoid body dissociation, all steps should be performed under sterile conditions.

▲ Partially dissociated EBs may adhere unspecifically to plastic surfaces such as the tube walls. Thus, avoid unnecessary rotation of tubes during dissociation and keep the EBs covered with enzyme solution at all times.

2.1 Reagent preparation

1. Prepare Enzyme A by reconstitution of the lyophilized powder in its vial with 1 mL of Buffer A. Do not vortex. Prepare aliquots of appropriate volume and avoid repeated freeze-thaw-cycles.

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Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution. For cell culture experiments subsequent to tissue dissociation, Enzyme A should be sterile filtered.

2. Prepare 1950 µL enzyme mix 1 by adding 50 µL of Enzyme P to 1900 µL of Buffer X and vortex. Pre-heat the mixture to 37 °C for 10–15 minutes before use.

   ▲ **Note:** For enumeration or magnetic cell separation of CD309$^+$ (KDR$^+$, Flk-1$^+$, VEGFR2) cells add 10 µL of Enzyme P to 1940 µL of Buffer X.

3. Prepare 30 µL enzyme mix 2 by adding 20 µL of Buffer Y to 10 µL of Enzyme A.

4. Add 30 µL enzyme mix 2 to 1950 µL enzyme mix 1.

Enzyme mix 1		Enzyme mix 2	
Enzyme P 50 µL	Buffer X 1900 µL	Buffer Y 20 µL	Enzyme A 10 µL

2.2 Automated dissociation using the gentleMACS™ Dissociator

▲ For details on the use of the gentleMACS™ Dissociator, refer to the gentleMACS Dissociator user manual.
The protocol is not compatible with the gentleMACS Octo Dissociator or the gentleMACS Octo Dissociator with Heaters.

2.2.1 Automated dissociation of up to 7 days old EBs

1. Transfer up to 20 mL of EB-containing cell culture medium into the gentleMACS M Tube. Centrifuge at 300×g for 2 minutes. Discard supernatant.
2. Add 10 mL of DPBS to the gentleMACS M Tube. Resuspend EBs carefully. Centrifuge at 300×g for 2 minutes. Discard supernatant.
3. Add 1980 µL of the combined pre-heated enzyme mix 1 and 2 to the gentleMACS M Tube and tightly close the lid.
4. Incubate sample for 10 minutes at 37 °C without agitation and ensure EBs are covered by the enzyme solution.
5. Invert the M Tube in one movement and attach it upside down onto the sleeve of the gentleMACS Dissociator. Keep the tube upside down during the following incubation and dissociation steps 6–9.
6. Run the gentleMACS Program **EB_01**.
7. Once the program is finished incubate sample for 5 minutes at 37 °C without agitation.
8. Attach M Tube upside down onto the sleeve of the gentleMACS Dissociator.
9. Run the gentleMACS Program **EB_02**.
10. Once the program is finished add 8 mL of cell culture medium or DPBS to the M Tube.
11. Resuspend sample and apply the cell suspension to a Pre-Separation Filter, 70 µm, placed on a suitable (13–15 mL) tube.

    ▲ **Note:** Dissociated EBs can be removed from the closed M Tube by pipetting through the septum-sealed opening in the center of the cap of the M Tube. Use ART 1000 REACH 1000 µL pipette tips.

12. Wash Pre-Separation Filter, 70 µm, with 3 mL of EB culture medium or DPBS.
13. Discard filter and centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
14. Resuspend cells with appropriate buffer or medium to the required volume for further applications.

2.2.2 Automated dissociation of 8–20 days old EBs

1. Transfer up to 20 mL of EB-containing cell culture medium into the gentleMACS M Tube. Centrifuge at 300×g for 2 minutes. Discard supernatant.
2. Add 10 mL of DPBS to the gentleMACS M Tube. Resuspend EBs carefully. Centrifuge at 300×g for 2 minutes. Discard supernatant.
3. Add 1980 µL of the combined pre-heated enzyme mix 1 and 2 to the gentleMACS M Tube and tightly close the lid.
4. Incubate sample for 10 minutes at 37 °C without agitation and ensure EBs are covered by the enzyme solution.
5. Invert the M Tube in one movement and attach it upside down onto the sleeve of the gentleMACS Dissociator. Keep the tube upside down during the following incubation and dissociation steps 6–12.
6. Run the gentleMACS Program **EB_01**.
7. Once the program is finished incubate sample for 10 minutes at 37 °C without agitation.
8. Attach M Tube upside down onto the sleeve of the gentleMACS Dissociator.
9. Run the gentleMACS Program **EB_02**.
10. Once the program is finished incubate sample for 5 minutes at 37 °C without agitation.
11. Attach M Tube upside down onto the sleeve of the gentleMACS Dissociator.
12. Run the gentleMACS Program **EB_02**.
13. Once the program is finished add 8 mL of cell culture medium or DPBS to the M Tube.
14. Resuspend sample and apply the cell suspension to a Pre-Separation Filter, 70 µm, placed on a suitable (13–15 mL) tube.

    ▲ **Note:** Dissociated EBs can be removed from the closed M Tube by pipetting through the septum-sealed opening in the center of the cap of the M Tube. Use ART 1000 REACH 1000 µL pipette tips.

15. Wash Pre-Separation Filter, 70 µm, with 3 mL of cultivation medium or DPBS.
16. Discard filter and centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
17. Resuspend cells with appropriate buffer or medium to the required volume for further applications.

2.3 Manual dissociation

▲ The protocol below is compatible with a wide range of EB-based differentiation protocols. However, depending on cell type of interest and the protocol used for EB generation, pipetting and incubation times may have to be optimized.

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▲ Dissociation by pipetting may give variable results, depending on the user. For optimal standardization, the use of the gentleMACS Dissociator is recommended (refer to section 2.2)

2.3.1 Manual dissociation of up to 7 days old EBs

1. Transfer up to 20 mL of EB-containing cell culture medium into a 50 mL tube. Centrifuge at 300×g for 2 minutes. Discard supernatant.
2. Add 10 mL of DPBS and resuspend EBs carefully. Centrifuge at 300×g for 2 minutes. Discard supernatant.
3. Add 1980 µL of the combined pre-heated enzyme mix 1 and 2.
4. Incubate for 10 minutes at 37 °C without agitation and ensure EBs are covered by the enzyme solution.
5. Start mechanical dissociation by carefully pipetting up and down using a 1000 µL pipette for 1 minute.
6. Incubate for 5 minutes at 37 °C without agitation.
7. Pipette up and down for 1 minute using a 1000 µL pipette.
8. Add 8 mL of cell culture medium or DPBS.
9. Pass the cell suspension through a Pre-Separation Filter, 70 µm, placed on a suitable (13–15 mL) tube.
10. Wash the Pre-Separation Filter with 3 mL of EB culture medium or DPBS.
11. Discard filter and centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
12. Resuspend in an appropriate buffer or medium required for your downstream application.

2.3.2 Manual dissociation of 8–20 days old EBs

1. Transfer up to 20 mL of EB-containing cell culture medium into a 50 mL tube. Centrifuge at 300×g for 2 minutes. Discard supernatant.
2. Add 10 mL of DPBS and resuspend EBs carefully. Centrifuge at 300×g for 2 minutes. Discard supernatant.
3. Add 1980 µL of the combined pre-heated enzyme mix 1 and 2.
4. Incubate sample for 10 minutes at 37 °C without agitation and ensure EBs are covered by the enzyme solution.
5. Start mechanical dissociation by carefully pipetting up and down using a 1000 µL pipette for 1 minute.
6. Incubate sample for 10 minutes at 37 °C without agitation.
7. Pipette up and down for 1 minute using a 1000 µL pipette.
8. Incubate for 5 minutes at 37 °C without agitation.
9. Pipette up and down for 1 minute using a 1000 µL pipette.
10. Add 8 mL of cell culture medium or DPBS.
11. Pass the cell suspension through a Pre-Separation Filter, 70 µm, placed on a suitable (13–15 mL) tube.
12. Wash the Pre-Separation Filter with 3 mL of EB culture medium or DPBS.
13. Discard filter and centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
14. Resuspend in an appropriate buffer or medium required for your downstream application.

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

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Miltenyi Biotec

Epidermis Dissociation Kit
human

Order no. 130-103-464

Contents

1. Description

Components	3 vials, containing **1 vial of Enzyme G (lyophilized powder)** **2.5 mL of Enzyme P** **1 vial of Enzyme A (lyophilized powder)**
Size	For 100 digestions. The specified number of digestions is valid for 4 mm biopsies following the protocol in chapter 2.2.
Storage	Upon arrival immediately store Enzyme P in aliquots at –20 °C. Store all other components at 2–8 °C upon arrival. 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 Epidermis Dissociation Kit

Human epidermal tissue can be dissociated to single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular adhesion proteins, which maintain the structural integrity of tissues.
In a first step, the epidermal cell layer is removed from the dermal cell layer after enzymatic treatment over night at 4 °C. The epidermal tissue is then further digested enzymatically and dissociated into a single-cell suspension by using the gentleMACS™ Dissociators.
Cells should be processed immediately for downstream applications, such as cell separation, cellular or molecular analyses.

1.2 Background information

The Epidermis Dissociation Kit, human enables the gentle and efficient generation of single-cell suspensions from human epidermal tissue. The kit has been particularly developed for the isolation of keratinocytes from diverse human skin biopsies. Furthermore, dissociated cells can be subsequently cultured or isolated using MACS® Technology.

1.3 Applications

- Dissociation of human skin tissue for the cultivation of keratinocytes.
- Phenotyping or enumeration of human epidermal cell populations by flow cytometry.

1.4 Reagent and instrument requirements

- RPMI 1640 (# 130-091-440)
- PBS: phosphate-buffered saline pH 7.2
- PB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA) by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with PBS. Keep buffer cold (2–8 °C).
- Pre-Separation Filter, 70 µm (# 130-095-823)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 4 °C
- gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) MACS Tissue Storage Solution (# 130-100-008)
- (Optional) Tool for taking punch biopsies (e.g., Biopsy Punch)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

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.

▲ The protocol has been optimized for the digestion of adult human skin from breast or abdominal reduction surgery.

▲ Up to five punch biopsies (4 mm each) can be used per digestion. When working with bigger punch biopsies, cut the biopsy into pieces with a maximum diameter of 4 mm.

2.1 Reagent and instrument preparation

▲ Prepare Enzyme G by reconstitution of the lyophilized powder in the vial with 3 mL of sterile, distilled water. Do not vortex. Produce aliquots of appropriate volume. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

▲ Prepare aliquots of appropriate volume of Enzyme P. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

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▲ Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL RPMI 1640. Do not vortex. Produce aliquots of appropriate volume. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

2.2 Epidermis dissociation protocol

2.2.1 Separation of epidermis from dermis (day l)

1. Wash human skin tissue in an appropriate buffer or cell culture medium, e.g., MACS Tissue Storage Solution.
2. Remove subcutaneous fat using scissors. If the diameter of the skin sample exceeds 4 mm in diameter, take one or more 4 mm diameter punches by rotating down the tool (e.g., Biopsy Punch) through epidermis and dermis. Store punches in an appropriate buffer or cell culture medium, e.g., MACS Tissue Storage Solution, until needed.
3. Transfer 1 mL of RPMI 1640 and 25 µL of Enzyme G to into a 2 mL tube and mix carefully. Keep on ice.
4. Transfer up to five samples of skin tissue (4 mm diameter) into the 2 mL tube.
5. Incubate sample for 14–18 hours at 4 °C using the MACSmix Tube Rotator (12 rpm).

2.2.2 Automated dissociation of epidermis using the gentleMACS™ Dissociator (day 2)

1. Prewarm the water bath to 37 °C.
2. Take the biopsy out of the 2 mL tube and peel off the epidermis from the dermis using curved tweezers.
3. (Optional) Transfer the epidermis without dermis back to the 2 mL tube and incubate for additional 2 hours at 37 °C.

   ▲ **Note:** This step will increase yield of Langerhans cells.
4. Prepare enzyme mix by adding 1 mL of RPMI 1640, 25 µL of Enzyme P, and 5 µL of Enzyme A into a new 2 mL tube. Keep on ice.

   ▲ **Note:** Do not mix Enzyme P and Enzyme A directly.
5. Transfer the epidermis into the 2 mL tube containing the enzyme mix.
6. Incubate for 60 minutes at 37 °C in a water bath.

   ▲ **Note:** It has to be ensured that the sample material is located in the enzyme mix during the incubation time.
7. Lay the epidermis directly on the rotator/stator of the gentleMACS™ C Tube. Transfer the remaining liquid of the 2 mL tube in the C Tube.

   ▲ **Note:** It has to be ensured that the sample material is located in the enzyme mix in the area of the rotor/stator before starting the gentleMACS Program as the epidermis might easily stick to the tube wall and won´t be dissociated.
8. Stop enzymatic reaction by adding 1 mL of cold PB buffer.
9. 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.
10. Run the gentleMACS Program **B**.
11. After termination of the program, detach C Tube from the gentleMACS Dissociator.
12. Perform a short centrifugation step to collect the sample material at the tube bottom.
13. Resuspend sample by pipetting up and down 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.
14. Wash the Pre-Separation Filter, 70 µm, with 1 mL of cold PB Buffer.

    ▲ **Note:** (Optional) To collect remaining cells in the C Tube add buffer first to the C Tube and then on top of the filter.
15. Discard the Pre-Separation Filter, 70 µm, and centrifuge sample at 300×g for 10 minutes at room temperature. Aspirate supernatant completely
16. Resuspend cells by pipetting up and down with PB buffer or an appropriate buffer to the required volume for further applications.

    ▲ **Note:** If cell clumps occur after the washing step, add another 10 µL of Enzyme A per mL of cell suspension, mix gently, incubate for 5 minutes at 37 °C in a water bath, and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely and repeat step 16.
17. Process cells immediately for further applications.

3. Appendix

Target cell yield per sample

Typical target cell yields per 4 mm punch biopsy of adult abdominal skin are about 8×10^4 total cells (mostly keratinocytes) and 3×10^3 Langerhans cells.

Typical staining

Epitopes which are intact after the dissociation procedure are, e.g., CD29 and CD49f (both positive for keratinocytes), CD45, CD207, CD1a, and HLA-DR (all positive for Langerhans cells).
Epitopes which are degraded after the dissociation procedure are, for example, CD117 (marker for melanocytes).

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

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140-004-332.02

Miltenyi Biotec

Epidermis Dissociation Kit

mouse

Order no. 130-095-928

Contents

1. Description

Components 4 vials, containing
13 mL of Buffer S (20× Stock Solution)
1 vial of Enzyme G (lyophilized powder)
2.5 mL of Enzyme P
1 vial of Enzyme A (lyophilized powder)

Size For 25 digestions.
The specified number of digestions is valid when digesting skin with an average weight of 1.5 g following the protocol in chapter 2.2.

Storage Upon arrival immediately store Enzyme P in aliquots at –20 °C. Store all other components at 2–8 °C upon arrival. Reconstitute Enzymes G and A 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 Epidermis Dissociation Kit

Epidermal tissue can be dissociated to single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular adhesion proteins which maintain the structural integrity of tissues.
In a first step the epidermal cell layer is removed from the dermal cell layer after enzymatic treatment over night at 4 °C.
The epidermal tissue is then further digested enzymatically and dissociated into a single-cell suspension by either using the gentleMACS™ Dissociators or by manual handling.
Cells should be processed immediately for downstream applications, such as cell separation, cellular or molecular analysis.

1.2 Background information

The Epidermis Dissociation Kit, mouse enables the gentle and efficient generation of single-cell suspensions from mouse epidermal tissue. The kit has been particulary developed for the isolation of epidermal Langerhans cells.

1.3 Applications

- Dissociation of mouse skin tissue for the isolation of Langerhans cells using the Epidermal Langerhans Cell MicroBead Kit (# 130-095-408).
- Phenotyping or enumeration of epidermal cell populations by flow cytometry.

1.4 Reagent and instrument requirements

- PBS: phosphate-buffered saline pH 7.2
- PB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA) by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with PBS. Keep buffer cold (2–8 °C).
- MACS SmartStrainers, 70 µm (# 130-098-462)
- (Optional) gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937)
- (Optional) 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. 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.

▲ The protocol is developed for the digestion of skin derived from 6–10 weeks old female C57 BL/6 or Balb/c mice.

▲ **Note:** It is not recommended to use skin derived from younger or older mice.

▲ An amount of up to 1.5 g skin tissue can be used per digestion, which corresponds approximately to the weight of back skin derived from two mice. When working with less than 1.5 g use the same volumes as indicated below.

140-003-088.04

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2.1 Reagent and instrument preparation

▲ Prepare 1× Buffer S by adding, for example, 1 mL of 20× Buffer S aseptically to 19 mL of sterile, distilled water. Store at 2–8 °C.

▲ **Note:** Handle under sterile conditions.

▲ Prepare Enzyme G by reconstitution of the lyophilized powder in the vial with 3 mL of sterile, distilled water. Do not vortex. Produce aliquots of appropriate volume. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

▲ Prepare aliquots of appropriate volume of Enzyme P. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

▲ Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL 1× Buffer S. Do not vortex. Produce aliquots of appropriate volume. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

2.2 Epidermis dissociation protocol

2.2.1 Separation of epidermis from dermis (day l)

1. Remove hair completely from the back of the mice by plucking against the growth direction of the hair.
2. Cut skin at the base of the tail with a transverse cut using scissors and incise then upwards on both sides. Peel the tissue off with tweezers and make a transverse cut at the neck.
3. Wash tissue by placing the skin in a 100 mm petri dish containing 20 mL of PBS buffer.
4. Determine weight of the tissue to make sure that the limit of 1.5 g per digestion is not exceeded.
5. Transfer tissue to a new 100 mm petri dish with the epidermal side facing downwards.
6. Carefully scrape off the subcutaneous fat using a scalpel.

7. Subdivide skin of one mouse in 2 pieces.
8. Prepare enzyme mix by adding 100 µL of Enzyme G to 3.9 of mL PBS buffer in a 50 mm petri dish. Mix well.
9. Place tissue pieces on top of the enzyme mix with the dermal side facing downwards.

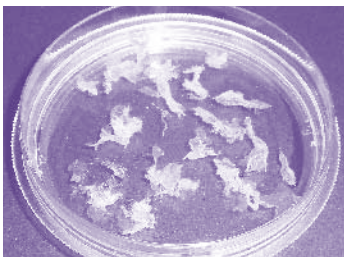
10. Incubate at 4 °C for 16 hours.

▲ **Note:** Do not extend incubation time!

2.2.2 Dissociation of epidermis (day 2)

1. Prewarm the water bath to 37 °C.
2. Peel off the epidermis from the dermis using curved tweezers.

3. Transfer the epidermis into a new 50 mm petri dish containing 4 mL of 1× Buffer S and cut into smaller pieces.



4. For manual dissociation proceed with chapter 2.2.2.1.

   For automatic dissociation using the gentleMACS Dissociator proceed with chapter 2.2.2.2.

2.2.2.1 Manual dissociation

1. Prepare enzyme mix by adding 3.9 mL of 1× Buffer S, 100 µL of Enzyme P, and 20 µL of Enzyme A into a 50 mL tube.
2. Transfer epidermis pieces into the tube containing the enzyme mix. The sample material must not stick to the wall of the tube.
3. Incubate for 20 minutes at 37 °C in a water bath.

▲ **Note:** It has to be ensured that the sample material is located in the enzyme mix during the incubation time.

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4. Stop enzymatic reaction by adding 4 mL of PB buffer.
5. Pour sample onto a MACS SmartStrainer, 70 µm, placed on a 50 mL tube. Pass sample through the 70 µm mesh by using a plunger of a 1 mL syringe.

   ▲ **Note:** It is not recommended to use a pipette tip to add the sample to the MACS SmartStrainer, 70 µm, as the tissue tends to stick to the pipette tip wall.
6. Wash the MACS SmartStrainer, 70 µm, with 5 mL of PB buffer and discard it.
7. Repeat steps 5 and 6 using a new MACS SmartStrainer, 70 µm.
8. Centrifuge sample at 300×g for 10 minutes at room temperature.
9. Aspirate supernatant completely and resuspend cells with PB buffer or an adequate buffer to the required volume for further applications.

   ▲ **Note:** Do not vortex.

   ▲ **Note:** If cell clumps occur after the washing step, add another 10 µL of Enzyme A per mL of cell suspension, mix gently and incubate for 5 minutes at 37 °C in a water bath, centrifuge at 300×g for 10 minutes and repeat step 9.
10. Process cells immediately for further applications.

2.2.2.2 Automated dissociation using the gentleMACS™ Dissociator

1. Prepare enzyme mix by adding 3.9 mL of 1× Buffer S, 100 µL of Enzyme P, and 20 µL of Enzyme A into a gentleMACS™ C Tube.
2. Transfer epidermis pieces into the gentleMACS C Tube containing the enzyme mix. The sample material must not stick to the wall of the tube.
3. Incubate for 20 minutes at 37 °C in a water bath.

   ▲ **Note:** It has to be ensured that the sample material is located in the enzyme mix during the incubation time.
4. Stop enzymatic reaction by adding 4 mL of PB buffer.
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 enzyme mix in the area of the rotor/stator before starting the gentleMACS Program.
6. Run the gentleMACS Program **B**.
7. After termination of the program, detach C Tube from the gentleMACS Dissociator.
8. Apply sample to a MACS SmartStrainer, 70 µm, 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.
9. Wash the MACS SmartStrainer, 70 µm, with 10 mL of PB Buffer.
10. Discard the MACS SmartStrainer, 70 µm, and centrifuge sample at 300×g for 10 minutes at room temperature.
11. Aspirate supernatant completely and resuspend cells with PB buffer or an adequate buffer to the required volume for further applications.

    ▲ **Note:** Do not vortex.

    ▲ **Note:** If cell clumps occur after the washing step, add another 10 µL of Enzyme A per mL of cell suspension, mix gently and incubate for 5 minutes at 37 °C in a water bath, centrifuge at 300×g for 10 minutes and repeat step 11.
12. Process cells immediately for further applications.

140-003-088.04

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

Warranty

Miltenyi Biotec

Lamina Propria Dissociation Kit
mouse

Order no. 130-097-410

Contents

1. Description

Components 5 vials, containing:

2 vials of Enzyme D (lyophilized powder)
1 vial of Enzyme R (lyophilized powder)
1 vial of Enzyme A (lyophilized powder)
1 vial of Buffer A

Size For 50 digestions.

The specified number of digestions is valid when digesting small intestine tissue up to a weight of 1 g following the protocol in chapter 2.2.

Storage Upon arrival immediately 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 Lamina Propria Dissociation Kit

Lamina propria tissue can be dissociated to single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular adhesion proteins which maintain the structural integrity of tissues.
In a first step, the intraepithelial lymphocytes (IELs) are disrupted from the mucosa by shaking the tissue in a predigestion solution.
Then, the lamina propria tissue is further treated enzymatically and mechanically dissociated into a single-cell suspension by using the gentleMACS™ Dissociators.
Cells should be processed immediately for downstream applications, such as MACS® MicroBead separations, cellular or molecular analysis.

1.2 Background information

The Lamina Propria Dissociation Kit, mouse has been developed for the gentle, rapid, and efficient generation of single-cell suspensions from mouse small intestine lamina propria tissue. It is optimized for a high yield of small intestine lamina propria lymphocytes, while preserving cell surface epitopes. Isolated cells can be analyzed directly or subjected to magnetic cell separation using MACS Technology to obtain specific cell subsets.

Dissociated cells can be analyzed *in vitro* or subpopulations of cells can be isolated using MACS Technology.

1.3 Applications

- Dissociation of mouse lamina propria tissue derived from 6–10 week old C57BL/6 or Balb/c mice into single-cell suspensions for subsequent cell separations using MACS Technology.
- Phenotyping or enumeration of lamina propria cell populations by flow cytometry.

1.4 Reagent and instrument requirements

- (Sterile) 1× Hank's balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+} containing 10 mM HEPES, in the following referred to as HBSS (w/o). Store at room temperature.
- (Sterile) 1× HBSS with Ca^{2+} and Mg^{2+} containing 10 mM HEPES, in the following referred to as HBSS (w). Store at room temperature.
- (Sterile) Predigestion solution: prepare 1× HBSS (w/o) containing 5 mM EDTA, 5% fetal bovine serum (FBS), 1 mM DTT freshly before each digestion. Store at room temperature.
  ▲ **Note:** Per digestion a volume of 40 mL of the predigestion solution is required.
- (Sterile) Digestion solution: prepare 1× HBSS (w) containing 5% FBS freshly before each digestion. Store at room temperature.
  ▲ **Note:** Per digestion a volume of 2.5 mL of the digestion solution is required.
- (Sterile) PB buffer: prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 0.5 % bovine serum albumin (BSA). Keep buffer cold (2–8 °C).
  ▲ **Note:** BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or FBS.
- MACS SmartStrainers, 100 µm (# 130-098-463)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C.
- 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) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

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2. Protocol

▲ For details on the use of the gentleMACS Dissociators, refer to the respective user manuals.

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ Volumes given below are for up to 1 g of starting tissue material, which is approximately the weight of a small intestine derived from one 6–10 week old C57BL/6 or Balb/c mouse. When working with less than 1 g, use the same volumes as indicated, but do not use less than 0.8 g for each digestion.

▲ Operate MACSmix Tube Rotator with continuous rotation at a speed of approximately 12 rpm.

2.1 Reagent preparation

1. Prepare Enzyme D by reconstitution of the lyophilized powder in each vial with 3 mL of sterile HBSS (w). 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 sterile HBSS (w). 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 of Buffer A supplied with the kit. 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 Lamina propria dissociation protocol

1. Remove intestine from a 6–10 week old C57BL/6 or Balb/c mouse and place it in HBSS (w/o) in a Petri dish.
2. Clear the intestine of feces by holding it with forceps and flushing with HBSS (w/o) using a syringe.
3. Remove residual fat tissue and Peyer's patches.
4. Cut the intestine first longitudinally and then laterally into pieces of approximately 0.5 cm length.
5. Transfer the tissue pieces into a 50 mL tube containing 20 mL of predigestion solution.
6. Incubate the sample for 20 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
7. Mix well for 10 seconds using a vortex mixer and apply the sample onto a MACS SmartStrainer, 100 µm, placed on a 50 mL tube.

   ▲ **Note:** Flow through contains intraepithelial lymphocytes (IELs). If IEL isolation is desired store flow-through on ice.
8. Transfer the lamina propria tissue pieces into a new 50 mL tube containing 20 mL of fresh predigestion solution.
9. Incubate the sample for 20 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
10. Mix well for 10 seconds using a vortex mixer and apply the sample onto a MACS SmartStrainer, 100 µm, placed on a 50 mL tube.

    ▲ **Note:** If IEL isolation is desired, combine the flow through, which contains IELs, with flow through from step 7. Store the cell suspension for at least 10 minutes on ice and transfer the supernatant that contains the cells then into a new tube.
11. Transfer the lamina propria tissue pieces into a new 50 mL tube containing 20 mL of HBSS (w/o).
12. Incubate the sample for 20 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.

    ▲ **Note:** In the meantime: Transfer 2.35 mL of digestion solution into a gentleMACS C Tube and pre-heat at 37 °C for 15 minutes.
13. Mix well for 10 seconds using a vortex mixer and apply the sample onto a MACS SmartStrainer, 100 µm, placed on a 50 mL tube.

    ▲ **Note:** If IEL isolation is desired, combine the flow through, which contains IELs, with supernatant from step 10. Centrifuge at 300×g for 10 minutes at room temperature. Aspirate supernatant completely and resuspend IELs with appropriate buffer to the required volume for further applications.
14. Prepare enzyme mix by adding 100 µL of Enzyme D, 50 µL of Enzyme R, and 12.5 µL of Enzyme A into a gentleMACS C Tube containing the pre-heated 2.35 mL of digestion solution and mix gently.
15. Transfer the intestine tissue into the gentleMACS C Tube containing the enzyme mix and close C Tube tightly.
16. (Optional) If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_m_LPDK_1** and continue with step 20.
17. Incubate the sample for 30 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
18. 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.
19. Run the gentleMACS Program **m_intestine_01**.
20. 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.
21. Resuspend sample, add 5 mL of PB buffer, and apply the cell suspension to a MACS SmartStrainer, 100 µm, 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.
22. Wash MACS SmartStrainer, 100 µm, with 10 mL of PB buffer.
23. Discard the MACS SmartStrainer, 100 µm, and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
24. Resuspend lamina propria lymphocytes with an appropriate buffer to the required volume for further applications, for example, resuspend cells in PB buffer for magnetic cell separation or flow cytometry.

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25. Cells should be processed immediately for further applications.
26. (Optional) To remove erythrocytes or dead cells, perform a density gradient centrifugation step.

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140-003-623.02

Miltenyi Biotec

Lung Dissociation Kit
mouse

Order no. 130-095-927

Contents

1. Description

This product is for research use only.

Components	4 vials, containing: **13 mL of Buffer S (20× Stock Solution)** **2 vials of Enzyme D (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)**
Size	For 50 digestions. The specified number of digestions is valid when digesting lung material of one mouse with an average weight of 110–150 mg 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 Lung Dissociation Kit

The lung tissue 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 lung 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 Lung Dissociation Kit, mouse has been designed for the gentle, rapid, and efficient generation of single-cell suspensions from mouse lung. It is optimized for a high yield of leukocytes and endothelial cells, while preserving all cell surface epitopes. Dissociated cells can be 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 lung tissue into single-cell suspensions for subsequent cell separations using MACS Technology.
- Cultivation of lung cell populations.
- Phenotyping or enumeration of lung cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- PBS: phosphate-buffered saline pH 7.2
- 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).
  Always use freshly prepared buffer. Do **not use** autoMACS® Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.
- MACS SmartStrainers (70 µm) (# 130-098-462)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C
- 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) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

140-003-085.05

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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.

▲ Lung material of one mouse is dissociated in a volume of approximately 2.5 mL enzyme mix. The weight of the lungs of one mouse amounts to 110–150 mg (female BALB/c mouse, 6–7 weeks old).

2.1 Reagent preparation

1. Prepare 1× Buffer S by adding, for example, 1 mL of 20× Buffer S aseptically to 19 mL of sterile, distilled water. Store at 2–8 °C.
   ▲ **Note:** Handle under sterile conditions.
2. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL of 1× Buffer S. 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.
3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL of 1× Buffer S. 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 Lung dissociation protocol

1. Prepare enzyme mix by adding 2.4 mL of 1× Buffer S, 100 µL of Enzyme D, and 15 µL of Enzyme A into a gentleMACS C Tube.
2. Perfuse lungs via the right ventricle to remove blood cells.
3. Dissect mouse lungs into single lobes and rinse lobes in a petri dish containing PBS, pH 7.2, to remove remaining blood.
   ▲ **Note:** Remove thymus, heart, efferent and afferent blood vessels, trachea, and connective tissue from the lung tissue.
4. Transfer lobes of the lungs of one mouse into the gentleMACS C Tube containing the enzyme mix.
   ▲ **Note:** Cell yields can be increased by injecting the enzyme into the tissue. Therefore inflate each lung lobe on a petri dish by injecting the enzyme solution slowly 1–5-times using a 25 G needle connetcted to a 1 mL syringe.
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_lung_01**.
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_m_LDK_1** and continue with step 11.
7. After termination of the program, detach C Tube from the gentleMACS Dissociator.
   ▲ **Note:** The lung lobes will not be completely dissociated after this step. In the unexpected event that the lobes are not dissociated at all, repeat steps 6 and 7.
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_lung_02**.
11. After termination of the program, detach C Tube from the gentleMACS Dissociator.
12. (Optional) Perform a short centrifugation step to collect the sample material at the tube bottom.
13. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (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.
14. Wash MACS SmartStrainer (70 µm) with 2.5 mL 1× Buffer S.
15. Discard the MACS SmartStrainer (70 µm) and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
16. Resuspend cells with medium or an appropriate buffer to the required volume for further applications. For example, resuspend cells in PEB buffer for magnetic cell separatiom or flow cytometry.
17. Process cells immediately for further applications.

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

Warranty

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Miltenyi Biotec

Neonatal Heart Dissociation Kit

mouse and rat

Order no. 130-098-373

Contents

1. Description

Components	7 vials, containing: **2.5 mL of Enzyme P** **2×50 mL of Buffer X** **1.7 mL of Buffer Y** **1 vial of Enzyme A (lyophilized powder)** **1.2 mL of Buffer A** **1 vial of Enzyme D (lyophilized powder)**
Size	For 25 digestions of 2.5 mL.
Storage	Upon arrival immediately store Enzyme P in aliquots at –20 °C. Store all other components at 2–8 °C upon arrival. Reconstitute Enzymes A and D 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 Neonatal Heart Dissociation Kit

Neonatal hearts from mice and rats 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 neonatal hearts are enzymatically digested using the kit components and the gentleMACS™ Dissociator is 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

Neonatal cardiomyocytes are widely used to study and understand the morphological, biochemical, and electrophysiological characteristics of the heart based on a broad spectrum of experiments, such as studies of contraction, ischaemia, hypoxia, and the toxicity of various compounds.

The Neonatal Heart Dissociation Kit, mouse and rat has been designed for the gentle, rapid, and effective generation of single-cell suspensions from mouse and rat hearts. It is optimized for a high yield of viable cardiomyocytes as well as non-myocytes, e.g., cardiac fibroblasts and endothelial cells. The single-cell suspension can be cultured and utilized for functional, genetic, or molecular studies.

1.3 Applications

- Dissociation of mouse and rat neonatal hearts into single-cell suspensions.
- Single-cell suspensions derived from neonatal hearts can be used for:
  - Purification of cardiomyocytes.
  - Enumeration and phenotyping of individual cardiac cell populations by flow cytometry or fluorescence microscopy.
  - Cultivation of beating cardiomyocytes or non-myocytes like cardiac fibroblasts and endothelial cells.

1.4 Reagent and instrument requirements

- Phosphate-buffered saline (PBS), pH 7.4
- Cell culture medium without fetal bovine serum (FBS), e.g., DMEM (# 130-091-437)
- Cell culture medium with FBS
- MACS SmartStrainers, 70 µm (# 130-098-462)
- 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)
- Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- PEB buffer: Prepare a solution containing PBS, pH 7.4, 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).

  Always use freshly prepared buffer. Do **not use** autoMACS Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.

  ▲ **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 mouse or rat serum albumin, mouse or rat serum, or FBS. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

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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.

2.1 Reagent and instrument preparation

1. Enzyme P is ready to use. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months.
2. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL Buffer A supplied with the kit. 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.
3. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL of cell culture medium without FBS, e.g., DMEM (# 130-091-437). 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.

Enzyme mix 1		Enzyme mix 2		
Enzyme P 62.5 µL	Buffer X 2300 µL	Buffer Y 25 µL	Enzyme A 12.5 µL	Enzyme D 100 µL

2.2 Neonatal heart dissociation protocol

▲ For mouse and rat postnatal day 0–3 (P0–P3).

▲ Volumes given below are for up to 20 neonatal mouse hearts or up to 10 neonatal rat hearts per C Tube and 2.5 mL of enzyme mix. When working with more hearts, scale up all reagent volumes and total volumes accordingly. Up to 20 neonatal rat hearts can be dissociated per C Tube with 5 mL of enzyme mix.

1. For mouse:
   Harvest neonatal mouse hearts and transfer into a 10 cm dish containing PBS. Utilizing forceps, carefully pump remaining blood out of the hearts. Cut vessels and remaining connective tissue away from the ventricles.

   For rat:
   Harvest neonatal rat hearts and transfer into a 10 cm dish containing PBS. Cut vessels and remaining connective tissue away from the ventricles. Cut each heart into small pieces (1–2 mm^3).
2. Preheat enzyme mix 1 for 5 minutes at 37 °C.

   ▲ **Note:** Preheating is not required if using the heating function of the gentleMACS Octo Dissociator with Heaters.
3. Add 2362.5 µL of enzyme mix 1 to 137.5 µL of enzyme mix 2.
4. Transfer harvested tissue into the gentleMACS C Tube.

   ▲ **Note:** To reduce the volume of washing medium within the tube let tissue settle down by gravity and remove supernatant carefully.
5. Add 2.5 mL of enzyme mix, tightly close the C Tube.

   ▲ **Note:** Close C Tube tightly beyond the first resistance.
6. Invert C Tube and place it with the cap down. To maximize cell recovery the C Tube should remain in this orientation until step 11.
7. (Optional) If using the heating function of the gentleMACS Octo Dissociator with Heaters attach C Tube upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters. Run program **37C_mr_NHDK_1** and continue with step 12.
8. Incubate sample without agitation for 15 minutes at 37 °C.
9. Attach C Tube onto the sleeve of the gentleMACS Dissociator.
10. Run the gentleMACS Program **mr_neoheart_01**.
11. Repeat steps 8–10 two times.
12. After termination of the program, detach C Tube from the gentleMACS Dissociator and add 7.5 mL of cell culture medium with FBS.
13. Resuspend sample and apply the cell suspension to a MACS SmartStrainer, 70 µm, placed on a suitable tube.
14. Wash MACS SmartStrainer, 70 µm, with 3 mL of cell culture medium with FBS.
15. Discard filter and centrifuge cell suspension at 600×g for 5 minutes. Aspirate supernatant completely.
16. Proceed with a red blood cell lysis (refer to section 2.3).

2.3 Red blood cell lysis

1. Resuspend cell pellet in 1 mL of PEB buffer and add 10 mL of 1× Red Blood Cell Lysis Solution to remove erythrocytes.
2. Incubate for maximal 2 minutes at room temperature (19–25 °C).
3. Centrifuge at 600×g for 5 minutes. Aspirate supernatant completely.
4. Add 15 µL of Enzyme A to 10 mL PBS in a fresh tube.
5. Resuspend cell pellet in 10 mL PBS containing Enzyme A.
6. Centrifuge at 600×g for 5 minutes. Aspirate supernatant completely.
7. Resuspend cells with appropriate buffer or medium to the required volume for further applications.

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.

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Miltenyi Biotec

Neural Tissue Dissociation Kits

Neural Tissue Dissociation Kit (P)	130-092-628
Neural Tissue Dissociation Kit (T)	130-093-231

Contents

1. Description

Components	**Neural Tissue Dissociation Kit (P)** 6 vials, containing: 2.5 mL of Enzyme P 2×50 mL of Buffer X (sterile) 1.5 mL of Buffer Y (sterile) 1 vial of Enzyme A (lyophilized powder) 1 mL of Buffer A or **Neural Tissue Dissociation Kit (T)** 6 vials, containing: 10 mL of Enzyme T 2×50 mL of Buffer X (sterile) 1.5 mL of Buffer Y (sterile) 1 vial of Enzyme A (lyophilized powder) 1 mL of Buffer A
Size	For 50 digestions of 2 mL.
Storage	Upon arrival store Enzyme T of the Neural Tissue Dissociation Kit (T) aliquoted at –20 °C. Store all other components at 2–8 °C upon arrival. The expiration date is indicated on the box label. For information about reconstitution and storage after reconstitution of the lyophilized component refer to chapter 2.1.

1.1 Principle of the Neural Tissue Dissociation Kits

Neural tissues can be dissociated to single-cell suspensions by enzymatic degradation of the extracellular adhesion proteins which maintain the structural integrity of tissues.
The neural tissue can be dissociated either using the gentleMACS™ Dissociator or manually using Pasteur pipettes. After the tissue has been cut into small pieces, a pre-warmed enzyme mix is added to the tissue pieces and incubated with agitation at 37 °C. The tissue is mechanically dissociated and the suspension is applied to a MACS® SmartStrainer (70 µm). Optionally, myelin can be removed using Myelin Removal Beads II, as it can interfere with subsequent flow cytometric analysis or cell separation using MACS® Technology. Cells should be processed immediately for downstream applications, such as cellular or molecular analyses or cell separations.

1.2 Background information

The Neural Tissue Dissociation Kits (NTDK) have been designed for the gentle but rapid and efficient generation of single-cell suspensions from neural tissues. In combination with the gentleMACS Dissociators, which provide optimized programs to attain single-cell suspensions from various neural tissues, they allow automated tissue dissociation in a closed, sterile system.

1.3 Applications

- Dissociation of neural tissues to single-cell suspensions for subsequent cell separations using MACS Technology, for example, isolation of microglia using CD11b (Microglia) MicroBeads, human and mouse (# 130-093-634) or isolation of astrocytes using the Anti-GLAST (ACSA-1) MicroBead Kit, human, mouse, and rat (# 130-095-826).
- Dissociation of subventricular zone (SVZ) tissue to single-cell suspensions for neurosphere assay.
- Dissociation of neural tissue for *in vitro* cultivation.
- Enumeration and phenotyping of individual neural cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- Hanks´ Balanced Salt Solution (HBSS) without Ca^{2+} and Mg^{2+} (Sigma-Aldrich # 55021C), in the following referred to as HBSS (w/o)
- HBSS with Ca^{2+} and Mg^{2+} (Sigma-Aldrich # 55037C), in the following referred to as HBSS (w)
- (Optional) Beta-mercaptoethanol, 50 mM
- 50 mL tubes
- MACS SmartStrainer (70 µm) (# 130-098-462) for 50 mL tubes
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubation oven at 37 °C
- (Optional) gentleMACS Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427), and C Tubes (# 130-093-237, # 130-096-334)

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- (Optional) MACS Neuro Medium (# 130-093-570)
- (Optional) (Optional) MACS NeuroBrew-21 (# 130-093-566)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.
- (Optional) Myelin Removal Beads II, human, mouse, rat (# 130-096-433, # 130-096-733).

Additional for manual dissociation:

- (Sterile) scalpel
- 35 mm diameter sterile petri dish
- (Sterile) glass Pasteur pipettes

Make sure the antigen epitope that is necessary for downstream applications is conserved during the dissociation procedure.

For a detailed list of antigen compatibilities and the right choice of NTDK refer to the table on NTDK product page at www.miltenyibiotec.com.

In case your epitope of interest is not listed please contact technical support. You can also perform a staining experiment with this antibody after using different enzyme concentrations, i.e., different dilutions of Enzyme P or T (e.g. for NTDK (P) 1:5, 1:10; for NTDK (T) 1:2.5) prior to isolation experiments to analyze the stability of your antibody epitope.

2. Protocol

2.1 Reagent and instrument preparation

▲ For optional dissociation of neural tissue in combination with the gentleMACS Dissociator, please refer to section 2.2.1 or 2.2.2. For manual dissociation of neural tissue refer to section 2.2.3.

▲ Volumes given below are for up to 400 mg of starting tissue material. When working with less than 400 mg, use the same volumes as indicated. Tissue quantities of 200 mg and less can be processed in a single 2 mL reaction tube. Tissue quantities of greater than 400 mg can be pooled and processed in an appropriate-sized conical tube. When working with more than 400 mg, scale up all reagent volumes and total volumes accordingly.

1. (Optional for increased stability of enzymes) Add beta-mercaptoethanol to Buffer X to a final concentration of 0.067 mM. For example, add 13.5 µL of 50 mM beta-mercaptoethanol to 10 mL of Buffer X.
   ▲ **Note:** This solution will then be stable for 1 month at 4 °C.
2. Resuspend the lyophilized powder in the vial labeled Enzyme A with 1 mL of Buffer A. Do **not** vortex.This solution should then be aliquoted and stored at –20 °C for later use.

	Enzyme mix 1		Enzyme mix 2	
NTDK (P)	Enzyme P 50 µL	Buffer X 1900 µL	Buffer Y 20 µL	Enzyme A 10 µL
NTDK (T)	Enzyme T 200 µL	Buffer X 1750 µL	Buffer Y 20 µL	Enzyme A 10 µL

2.2 Neural tissue dissociation protocols

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ In case of subsequent gene expression profiling perform all steps at 4 °C instead of room temperature.

▲ These protocols describe the dissociation of mouse brain tissue, though, in principle, they are transferable to other neural tissue types.

▲ The MACSmix Tube Rotator is used with continuous rotation at a speed of approximal 4 rpm.

2.2.1 Automated dissociation using the gentleMACS™ Dissociator or the gentleMACS™ Octo Dissociator

▲ For details on the use of the gentleMACS™ Dissociators, refer to the respective user manual.

▲ A maximum of 1600 mg mouse brain per C Tube can be processed. The total volume should not exceed 10 mL, minimum volume is 2 mL.

1. Remove the mouse brain. Determine the weight of tissue in 1 mL of HBSS (w/o).
2. Transfer the appropriate volume of enzyme mix 1 (refer to table in section 2.1) into a gentleMACS C Tube and pre-heat at 37 °C for 10–15 minutes before use.
3. Transfer mouse brain into the C Tube containing 1950 µL of the pre-heated enzyme mix 1 per up to 400 mg of tissue.
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 to the upper right of the rotor blade.
5. Run the gentleMACS Program **m_brain_01.**
6. Incubate sample for 15 minutes at 37 °C under slow, continuous rotation using the MACSmix Tube Rotator.
7. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.
8. Run the gentleMACS Program **m_brain_02.**
9. Prepare 30 µL enzyme mix 2 per up to 400 mg tissue by adding 20 µL of Bufer Y to 10 µL of Enzyme A.
10. Transfer enzyme mix 2 into the C Tube. Invert gently to mix. Do not vortex.
    ▲ **Note:** Enzyme mix can be added into the closed C Tube by pipetting through the septum-sealed opening in the center of the cap of the C Tube. Use 10–200 µL pipette tips.
11. Incubate sample for 10 minutes at 37 °C under slow, continuous rotation using the MACSmix Tube Rotator.
12. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.
13. Run the gentleMACS Program **m_brain_03.**
14. Incubate sample for 10 minutes at 37 °C under slow, continuous rotation using the MACSmix Tube Rotator.
15. (Optional) Centrifuge briefly to collect the sample at the bottom of the tube.

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16. Resuspend sample and apply the cell suspension to a MACS SmartStrainer, 70 µm, placed on a 50 mL tube.

    ▲ **Note:** When upscaling the reagent volume and total volumes, increase also the number of MACS SmartStrainers (70 µm). One MACS SmartStrainer (70 µm), can be used for up to 2 mL.

    ▲ **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.

    ▲ **Note:** Cells with a diameter >70 µm may be lost. To obtain these cells within the flow through, use a cell strainer with an appropriate mesh size.

17. Apply 10 mL of HBSS (w) through MACS SmartStrainer, 70 µm.

    ▲ **Note:** When working with more than 400 mg mouse brain wash MACS SmartStrainer (70 µm), with an appropriate amount of HBSS (w), five times the enzyme solution volume. If necessary split the sample.

18. Discard MACS SmartStrainer, 70 µm, and centrifuge cell suspension at 300×g for 10 minutes at room temperature Aspirate supernatant completely.

19. (Optional) Resuspend cell suspension in 10 mL HBSS (w) and centrifuge at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.

20. Resuspend cells with buffer to the required volume for further applications.

    ▲ **Note:** If problems with the formation of a compact pellet occur after either washing step, add another 30 µL of enzyme mix 2 per mL of cell suspension, mix gently, and incubate for a minimum of 5 minutes at 37 °C using the MACSmix Tube Rotator.

21. (Optional) For myelin removal, use Myelin Removal Beads II. For details refer to the Myelin Removal Beads II data sheet.

    ▲ **Note:** Myelin removal is recommended when working with brain tissue of mice or rat older than P7 as well as human tissue.

22. Cells should be processed immediately for further applications.

2.2.2 Automated dissociation using the gentleMACS™ Octo Dissociator with Heaters

▲ For details on the use of the gentleMACS™ Octo Dissociator with Heaters, refer to the user manual.

▲ A maximum of 1600 mg mouse brain per C Tube can be processed. The total volume should not exceed 10 mL, minimum volume is 2 mL.

▲ For microglia isolation from adult mouse brain, use theNTDK (P) with the following protocol and the gentleMACS Program **37C_ABDK**.

1. Remove the mouse brain. Determine the weight of tissue in 1 mL of HBSS (w/o).
2. Transfer the appropriate volume of enzyme mix 1 (refer to table in section 2.1) into a gentleMACS C Tube
3. Transfer mouse brain into the C Tube containing 1950 µL of enzyme mix 1 per up to 400 mg of tissue.
4. Transfer 30 µL enzyme mix 2 into the C Tube.
5. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters.

    ▲ **Note:** It has to be ensured that the sample material is located to the upper right of the rotor blade.

6. Run the gentleMACS Program **37C_NTDK_1**.

    ▲ **Note:** For microglia isolation from adult mouse brain use **37C_ABDK**.

7. (Optional) Centrifuge briefly to collect the sample at the bottom of the tube.
8. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (70 µm), placed on a 50 mL tube.

    ▲ **Note:** When upscaling the reagent volume and total volumes, increase also the number of filters. One filter can be used for up to 2 mL.

    ▲ **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.

    ▲ **Note:** Cells with a diameter >70 µm may be lost. To obtain these cells within the flow through, use a cell strainer with an appropriate mesh size.

9. Apply 10 mL of HBSS (w) through the MACS SmartStrainer.

    ▲ **Note:** When working with more than 400 mg mouse brain wash cell strainer with an appropriate amount of HBSS (w), five times the enzyme solution volume. If necessary split the sample.

10. Discard the MACS SmartStrainer and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
11. (Optional) Resuspend cell suspension in 10 mL HBSS (w) and centrifuge at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
12. Resuspend cells with buffer to the required volume for further applications.

    ▲ **Note:** If problems with the formation of a compact pellet occur after either washing step, add another 30 µL of enzyme mix 2 per mL of cell suspension, mix gently, and incubate for a minimum of 5 minutes at 37 °C using the MACSmix Tube Rotator.

13. (Optional) For myelin removal, use Myelin Removal Beads II. For details refer to the Myelin Removal Beads II data sheet.

    ▲ **Note:** Myelin removal is recommended when working with brain tissue of mice or rat older than P7 as well as human tissue.

14. Cells should be processed immediately for further applications.

2.2.3 Manual dissociation

1. Fire-polish three glass Pasteur pipettes so that decreasing tip diameters are achieved. For details refer to 4. Appendix.
2. Prepare 1950 µL enzyme mix 1 for up to 400 mg tissue (refer to table in section 2.1) and vortex. Pre-heat the mixture at 37 °C for 10–15 minutes before use.
3. Remove the mouse brain. Determine the weight of tissue in 1 mL of cold HBSS (w/o) to make sure the 400 mg limit per digestion is not exceeded.
4. Place the brain on the lid of a 35 mm diameter petri dish, remove the meninges (optional), and cut brain into small pieces using a scalpel.

    ▲ **Note:** For certain applications such as cultivation of neuronal cells, meninges should be removed.

5. Using a 1 mL pipette tip, add 1 mL of HBSS (w/o) and pipette pieces back into an appropriate-sized tube. Rinse with HBSS (w/o).

    ▲ **Note:** When using <200 mg of brain tissue, return pieces to a 2 mL reaction tube. For tissue quantities >200 mg, pipette pieces into a 15 mL conical tube.

    ▲ **Note:** When working with mice older than P10, cut 2–4 mm off the end of the pipette tip to facilitate pipetting.

6. Centrifuge at 300×g for 2 minutes at room temperature and aspirate the supernatant carefully.
7. Add 1950 µL of pre-heated enzyme mix 1 (Enzyme P or T and Buffer X) per up to 400 mg tissue.

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8. Incubate in closed tubes for 15 minutes at 37 °C under slow, continuous rotation using the MACSmix Tube Rotator.
9. Prepare 30 µL enzyme mix 2 per tissue sample by adding 20 µL of Buffer Y to 10 µL of Enzyme A (refer to table in section 2.1). Then add to sample.
10. Invert gently to mix. Do **not** vortex.
11. Dissociate tissue mechanically using the wide-tipped, fire-polished Pasteur pipette by pipetting up and down 10 times slowly. Avoid forming air bubbles.

    ▲ **Note:** If the pipette is blocked by tissue pieces, repeat this step once or twice.
12. Incubate at 37 °C for 10 minutes using a MACSmix Tube Rotator.
13. Dissociate tissue mechanically using the other two fire-polished pipettes in decreasing diameter. Pipette slowly up and down 10 times with each pipette, or as long as tissue pieces are still observable. Be careful to avoid the formation of air bubbles.
14. Incubate at 37 °C for 10 minutes using a MACSmix Tube Rotator.
15. Apply the cell suspension to a MACS SmartStrainer, 70 µm, placed on a 50 mL tube.

    ▲ **Note:** When upscaling the reagent volume and total volumes, increase also the number of MACS SmartStrainers, 70 µm. One MACS SmartStrainer (70 µm), can be used for up to 2 mL.

    ▲ **Note:** Cells with a diameter >70 µm may be lost. To obtain these cells within the flow through, use a cell strainer with an appropriate mesh size.
16. Apply 10 mL of HBSS (w) through MACS SmartStrainer, 70 µm.

    ▲ **Note:** When working with more than 400 mg mouse brain wash MACS SmartStrainer (70 µm), with an appropriate amount of HBSS (w), five times the enzyme solution volume. If necessary split the sample.
17. Discard MACS SmartStrainer, 70 µm, and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
18. (Optional) Resuspend cell suspension in 10 mL HBSS (w) and centrifuge at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
19. Resuspend cells with buffer to the required volume for further applications.

    ▲ **Note:** If problems with the formation of a compact pellet occur after either washing step, add another 30 µL of enzyme mix 2 per mL of cell suspension, mix gently, and incubate for a minimum of 5 minutes at 37 °C using the MACSmix Tube Rotator.
20. (Optional) For myelin removal, use Myelin Removal Beads II. For details refer to the Myelin Removal Beads II data sheet.

    ▲ **Note:** Myelin removal is recommended when working with brain tissue of mice or rat older than P7 as well as human tissue.
21. Cells should be processed immediately for further applications.

3. References

1. Lee, J.K. *et al.* (2008) Regulator of G-protein signaling 10 promotes dopaminergic neuron survival via regulation of the microglial inflammatory response. J. Neurosci. 28: 8517–8528.
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3. Nguyen, V. and McQuillen, P.S. (2010) AMPA and metabotropic excitoxicity explain subplate neuron vulnerability. Neurobiol. Dis. 37(1): 195–207.
4. Szulwach, K.E. *et al.* (2010) Cross talk between microRNA and epigenetic regulation in adult neurogenesis. J. Cell Biol. 189: 127–141.
5. Ganz, J. *et al.* (2010) Heterogeneity and Fgf dependence of adult neural progenitors in the zebrafish telencephalon. Glia 58(11): 1345–1363.

4. Appendix: Tips & hints

▲ For up-to-date information regarding antigen compatibilities of Neural Tissue Dissociation Kits for subsequent MACS Cell Separations, please refer to www.miltenyibiotec.com.

Production of appropriate Pasteur pipettes
For the manual dissociation protocol, three Pasteur pipettes with openings of decreasing diameter are needed. The opening of the first pipette should be rounded without significant decrease in the size of the opening. The smallest opening should not be smaller than 0.5 mm so that the cells are not forced through with too much pressure. To produce openings that get progressively smaller, rotate the pipettes quickly in the flame to fire-polish them for a few seconds. Production is easier if you apply the rubber sucker. Too much time may fuse the opening. The edges should be rounded.

Yield of viable cells is too low (dissociation is insufficient)
Make sure that the tissue pieces are agitated sufficiently during the entire time of incubation and do not stick to the bottom of the tube. Flick or invert the tube after adding the enzyme mixes if it is necessary. During the working steps at 37 °C the MACSmix Tube Rotator is convenient for this purpose. Apply the suspension to a cell strainer with a pore size appropriate for the size of the target cells.

Formation of a pellet after washing is inhibited by sticky threads or particles
Add another 30 µL enzyme mix 2 (Buffer Y and Enzyme A) per 2 mL and incubate for 5–10 minutes at 37 °C.

Single-cell suspension contains many dead cells
Make sure that the openings of the Pasteur pipettes are not to small. Pipette more slowly and do not vortex the cells. Avoid forming bubbles. Follow the protocol non-stop.

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

Warranty

Miltenyi Biotec

Skeletal Muscle Dissociation Kit
mouse and rat

Order no. 130-098-305

Contents

1. Description

This product is for research use only.

Components	7 vials, containing: **2 vials of Enzyme D (lyophilized powder)** **2.5 mL of Enzyme P** **2 vials of Enzyme A (lyophilized powder)** **2× 1 mL of Buffer A**
Size	For 50 digestions of 2.5 mL. The specified number of digestions is valid when digesting tissue up to 0.5 g following the protocol in chapter 2.2.
Storage	Upon arrival immediately store Enzyme P in aliquots at –20 °C. All other vials can be stored at 2–8 °C and have to be reconstituted 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 Skeletal Muscle Dissociation Kit

Mouse or rat skeletal muscle 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 skeletal muscle tissue is enzymatically digested using the kit components and the gentleMACS™ Dissociator is 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 Skeletal Muscle Dissociation Kit, mouse and rat has been developed for the gentle, rapid, and effective generation of single-cell suspensions from murine and rat skeletal muscle tissue. It is optimized for a high yield of viable cells, while preserving cell surface epitopes. Mature myotubes will be destroyed. The single-cell suspension can be analyzed *in vitro* for phenotype distributions, and other functional, genetic, or proteomic studies can be performed. Furthermore, dissociated cells can be subsequently cultured or isolated using MACS® Technology.

1.3 Applications

- Dissociation of mouse and rat skeletal muscle tissue into single-cell suspensions for subsequent cell separations using MACS Technology.
- Cultivation of muscle resident cell populations.
- Enumeration and phenotyping of cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- Cell culture medium without fetal bovine serum (FBS), e.g., DMEM
- MACS SmartStrainers (70 µm) (# 130-098-462)
- 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)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C
- 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).
  Always use freshly prepared buffer. Do **not use** autoMACS Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.

  ▲ **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 mouse or rat serum albumin, mouse or rat serum, or FBS. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

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140-003-773.05

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.

▲ Dissociation of up to 0.5 g tissue should be done in 2.5 mL; when working with 0.5–1.0 g of tissue dissociation should be done in 5 mL, scale up all reagent volumes and total volumes accordingly. If more than 1.0 g of tissue has to be digested it is recommended to use additional tubes.

▲ 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 each vial with 3 mL of 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. Enzyme P is ready to use. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months.
3. Prepare one vial of Enzyme A by reconstitution of the lyophilized powder with 1 mL Buffer A, supplied with the kit. 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.

   ▲ **Note:** If using the Satellite Cell Isolation Kit subsequently to skeletal muscle dissociation reconstitute the second vial of Enzyme A the same way.

2.2 Skeletal muscle dissociation protocol

1. Prepare enzyme mix by adding 2.35 mL of DMEM, 100 µL of Enzyme D, 25 µL of Enzyme P, and 18 µL of Enzyme A into a gentleMACS C Tube.
2. Cut skeletal muscle tissue into small pieces of 2–4 mm.
3. Transfer the tissue into the gentleMACS C Tube containing the enzyme mix and close it tightly.
4. (Optional) If using the heating function of the gentleMACS Octo Dissociator with Heaters attach C Tube upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters. Run program **37C_mr_SMDK_1** and continue with step 12.

   ▲ **Note:** To further increase the yield of satellite cells run program **37C_mr_SMDK_2** and continue with step 12.
5. Incubate sample for 30 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.

   ▲ **Note:** To further increase the yield of satellite cells extend the incubation time to 60 minutes at 37 °C.
6. 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.
7. Run the gentleMACS Program **m_muscle_01**.
8. After termination of the program, detach C Tube from the gentleMACS Dissociator.
9. Incubate sample for 30 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
10. 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.
11. Run again the gentleMACS Program **m_muscle_01**.
12. (Optional) Perform a short centrifugation step to collect the sample material at the tube bottom.
13. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (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.
14. Wash MACS SmartStrainer (70 µm) with 10 mL of DMEM.
15. Discard MACS SmartStrainer (70 µm) and centrifuge cell suspension at 300×g for 20 minutes. Aspirate supernatant completely.
16. Resuspend cells with an appropriate buffer to the required volume for further applications, for example, resuspend cells in PEB buffer for magnetic cell separation or flow cytometry.
17. (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.**

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Miltenyi Biotec

Spleen Dissociation Kit
mouse

Order no. 130-095-926

Contents

1. Description

Components	3 vials, containing: **13 mL of Buffer S (20× Stock Solution)** **1 vial of Enzyme D (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)**
Size	For 50 digestions. The specified number of digestions is valid when digesting a spleen with an average weight of 80–140 mg 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 Spleen Dissociation Kit

Spleen 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 spleen 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 Spleen Dissociation Kit, mouse has been designed for the gentle, rapid, and efficient generation of single-cell suspensions from mouse spleen. It is optimized for a high yield of leukocytes, especially dendritic cells, while preserving all cell surface epitopes. Dissociated cells can be 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 mouse spleen into single-cell suspensions for subsequent isolation of dendritic cells using CD11c MicroBeads, mouse (# 130-052-001), Anti-mPDCA-1 MicroBeads, mouse (# 130-091-965), or Pan DC MicroBeads, mouse (# 130-092-465).
- Cultivation of spleen cell populations.
- Phenotyping or enumeration of spleen cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- PBS: phosphate-buffered saline pH 7.2
- 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). Always use freshly prepared buffer. Do not use autoMACS® Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.
- Pre-Separation Filters, 30 µm (# 130-041-407)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C
- 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) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

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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.

▲ One mouse spleen is dissociated in a volume of approximately 2.5 mL enzyme mix. The weight of one mouse spleen amounts to 80–140 mg (female BALB/c mouse, 6–7 weeks old).

▲ Remove fat tissue from the dissected mouse spleen before dissociation.

2.1 Reagent preparation

1. Prepare 1× Buffer S by adding, for example, 1 mL of 20× Buffer S aseptically to 19 mL of sterile, distilled water. Store at 2–8 °C.
   ▲ **Note:** Handle under sterile conditions.
2. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL of 1× Buffer S. 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.
3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL 1× Buffer S. 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 Spleen dissociation protocol

1. Prepare enzyme mix by adding 2.4 mL of 1× Buffer S, 50 µL of Enzyme D, and 15 µL of Enzyme A into a gentleMACS C Tube.
2. Transfer one mouse spleen into the gentleMACS C Tube containing the enzyme mix.
3. 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.
4. Run the gentleMACS Program **m_spleen_02**.
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_m_SDK_1** and continue with step 9.
5. After termination of the program, detach C Tube from the gentleMACS Dissociator.
   ▲ **Note:** The spleen will not be completely dissociated after this step. In the unexpected event that the spleen is not dissociated at all, repeat steps 4 and 5.
6. Incubate sample for 15 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
   ▲ **Note:** Operate MACSmix Tube Rotator on permanent run at a speed of approximately 12 rpm.
7. 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.
8. Run the gentleMACS Program **m_spleen_03**.
9. After termination of the program, detach C Tube from the gentleMACS Dissociator.
10. (Optional) Perform a short centrifugation step to collect the sample material at the tube bottom.
11. Resuspend sample and apply the cell suspension to a Pre-Separation Filter, 30 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 the filter with 2.5 mL 1× Buffer S.
13. Discard the filter and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
14. Resuspend cells with medium or an appropriate buffer to the required volume for further applications. For example, resuspend cells in PEB buffer for magnetic cell separatiom or flow cytometry.
15. Process cells immediately for further applications.

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

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Miltenyi Biotec

Tumor Dissociation Kit
human

Order no. 130-095-929

Contents

1. Description

This product is for research use only.

Components	5 vials, containing: **2 vials of Enzyme H (lyophilized powder)** **1 vial of Enzyme R (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)** **1 mL of Buffer A**
Size	For 25 digestions. The specified number of digestions is valid when digesting a tumor in a range of 0.01–1 g following the protocol in chapter 2.2.
Storage	Upon arrival immediately 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 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. For detailed information about marker preservation, please contact Technical Support at macstec@miltenyibiotec.com.

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 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

- RPMI 1640 (# 130-091-440) or DMEM (# 130-091-437)
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- MACS SmartStrainers (70 µm) (# 130-098-462)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C.
- 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) MACS Tissue Storage Solution (# 130-100-008)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

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 0.01–1 g is dissociated in a volume of approximately 5 mL enzyme mix.

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

2.1 Reagent preparation

1. Prepare Enzyme H 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 H 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 of Buffer A supplied with the kit. 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

2.2.1 Dissociation of soft tumors

▲ For example, dissociation of melanoma, ovarian, colon, hypopharyngeal, or renal tumors.

1. Prepare enzyme mix by adding 4.7 mL of RPMI 1640 or DMEM, 200 µL of Enzyme H, 100 µL of Enzyme R, and 25 µL of Enzyme A 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**.
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_h_TDK_1** and continue with step 14.
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 a MACS SmartStrainer (70 µm) 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 MACS SmartStrainer (70 µm) with 20 mL of RPMI 1640 or DMEM.
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.

2.2.2 Dissociation of medium tumors

▲ For example, dissociation of lung and prostate tumors.

1. Prepare enzyme mix by adding 4.7 mL of RPMI 1640 or DMEM, 200 µL of Enzyme H, 100 µL of Enzyme R, and 25 µL of Enzyme A 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**.
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_h_TDK_2** and continue with step 14.
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.

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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 a MACS SmartStrainer (70 µm) 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 MACS SmartStrainer, 70 µm, with 20 mL of RPMI 1640 or DMEM.
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, pancreatic, hepatocellular, or head and neck squamous cell (HNSCC) tumors.

1. Prepare enzyme mix by adding 4.7 mL of RPMI 1640 or DMEM, 200 µL of Enzyme H, 100 µL of Enzyme R, and 25 µL of Enzyme A 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**.
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_h_TDK_3** and continue with step 14.
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.
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 a MACS SmartStrainer (70 µm) 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 MACS SmartStrainer (70 µm) with 20 mL of RPMI 1640 or DMEM.
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.

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

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Miltenyi Biotec

Tumor Dissociation Kit
mouse

Order no. 130-096-730

Contents

1. Description

This product is for research use only.

Components	5 vials, containing: **2 vials of Enzyme D (lyophilized powder)** **1 vial of Enzyme R (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)** **1 mL of Buffer A**
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 2–8 °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

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. For detailed information about marker preservation, please contact Technical Support at macstec@miltenyibiotec.com.

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

- RPMI 1640 (# 130-091-440) or DMEM (# 130-091-437)
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- MACS SmartStrainers (70 µm) (# 130-098-462)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C.
- 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) MACS Tissue Storage Solution (# 130-100-008)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

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.

▲ Operate MACSmix Tube Rotator with continuous rotation at a speed of approximately 12 rpm.

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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 of Buffer A supplied with the kit. 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

2.2.1 Dissociation of soft and medium tumors

▲ For example, dissociation of melanoma (induced by B16 cell line) or colon (induced by CT26 cell line) tumors.

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.
2. Cut the tumor 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.**
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_m_TDK_1** and continue with step 10.
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.**
10. (Optional) 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 MACS SmartStrainer (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 MACS SmartStrainer (70 µm) with 10 mL of RPMI 1640 or DMEM.
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.

2.2.1 Dissociation of tough tumors

▲ For example, dissociation of breast (induced by 4T1 cell line) or lung (induced by TC1 cell line) tumors.

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.
2. Cut the tumor 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.**
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_m_TDK_2** and continue with step 10.
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** twice.
10. (Optional) Some larger pieces of tissue may remain. To further increase the cell yield allow the remaining tissue to settle and remove 1.5 mL of the supernatant to a fresh tube. Insert the C Tube with the remaining tissue pieces onto the sleeve of the gentleMACS Dissociator and run program **m_imptumor_01.** Combine the resulting cell suspension with the previously removed supernatant.
11. (Optional) 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.
12. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (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.
13. Wash MACS SmartStrainer (70 µm) with 10 mL of RPMI 1640 or DMEM.

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14. Centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant completely.
15. Resuspend cells with an appropriate buffer to the required volume for further applications.
16. (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

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