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

Components	**Brain Tumor Dissociation Kit (P)** 6 vials, containing: 1.25 mL of Solution 1 2×50 mL of Solution 2 (sterile) 1.5 mL of Solution 3 (sterile) 1 vial Solution 4 (45,000 U/mL) 1 mL of Storage Buffer for Solution 4 or **Brain Tumor Dissociation Kit (T)** 6 vials, containing: 4 mL of Solution 1 2×50 mL of Solution 2 (sterile) 1.5 mL of Solution 3 (sterile) 1 vial Solution 4 (45,000 U/mL) 1 mL of Storage Buffer for Solution 4
Size	For 25 digestions of 4 mL.
Storage	Store Solution 1 of the Brain Tumor Dissociation Kit (T) aliquoted at –20 °C upon arrival. Store all other components at 2–8 °C upon arrival. The expiration date is indicated on the kit box label. For information about reconstitution and storage after reconstitution of the lyophilized 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, 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
- (Sterile) cell strainer (40 µm mesh size, 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) or gentleMACS Octo Dissociator (# 130-095-937) 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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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. 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 Solution 2 to a final concentration of 0.067 mM. For example, add 13.5 µL of 50 mM beta-mercaptoethanol to 10 mL of Solution 2.
   ▲ **Note:** This solution will then be stable for 1 month at 4 °C.
2. Resuspend the lyophilized powder in the vial labeled Solution 4 with 1 mL of Storage Buffer for Solution 4. 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)	Solution 1 50 µL	Solution 2 3890 µL	Solution 3 40 µL	Solution 4 20 µL
BTDK (T)	Solution 1 160 µL	Solution 2 3780 µL	Solution 3 40 µL	Solution 4 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™ Dissociator

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

▲ A maximum of 1600 mg mouse 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 Solution 2 (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 the tissue into the C Tube containing the pre-heated Solution 2.
   ▲ **Note:** If very strong tissue is used, then cut it first into smaller pieces using a scalpel.
4. Add the appropriate volume of Solution 1, Solution 3, and Solution 4 (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**.
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 40 µm cell strainer, 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 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 >40 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 cell strainer.
    ▲ **Note:** When working with more than 800 mg mouse brain wash cell strainer with an appropriate amount of HBSS (w), five times the enzyme solution volume. If necessary split the sample.
17. Discard cell strainer 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.

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 Solution 2 (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.

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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 Solution 2 per 800 mg tissue.
8. Add the appropriate volume of Solution 1, Solution 3, and Solution 4 (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 40 µm cell strainer, 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 4 mL.

    ▲ **Note:** Cells with a diameter >40 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 cell strainer.

    ▲ **Note:** When working with more than 800 mg mouse brain wash cell strainer with an appropriate amount of HBSS (w), five times the enzyme solution volume. If necessary split the sample.
15. Discard cell strainer 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.

4. 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.

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

Warranty
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