Miltenyi Biotec

Brain Tumor Dissociation Kit (P)
human and mouse

Order no. 130-095-942

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 (lyophilized powder) 1 mL of Buffer A
Size	For 25 digestions of 4 mL.
Storage	Upon arrival immediately store Enzyme N of the Brain Tumor Dissociation Kit (P) aliquoted at –20 °C. Reconstitute Enzyme A before the date indicated on the vial label. 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

Brain tumor tissues can be dissociated into single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular matrix.

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. Optionally, myelin can be removed using Myelin Removal Beads II for improved results in downstream applications, such as molecular and flow cytometric analysis or cell separation.

1.2 Background information

The Brain Tumor Dissociation Kit (P) and the corresponding gentleMACS Programs have been designed for a gentle but rapid and efficient generation of single-cell suspensions from human and mouse 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.

When working with immune cells from human, mouse, or xenograft tumor, it is recommend to use the Tumor Dissociation Kit, human or mouse, respectively.

1.3 Applications

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

1.4 Reagent and instrument requirements

- HBSS with Ca^{2+} and Mg^{2+} (Sigma-Aldrich # 55037C), in the following referred to as HBSS (w)
- 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 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.
- (Optional) Myelin Removal Beads II, human, mouse, rat (# 130-096-733, # 130-096-433)
- (Optional) MACS Tissue Storage Solution (# 130-100-008)

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2. Protocol

2.1 Reagent and instrument preparation

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

▲ 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. When working with more than 800 mg, scale up all reagent volumes and total volumes, accordingly.

▲ A maximum of 1600 mg of tissue material can be processed per C Tube .

▲ The total volume should not exceed 10 mL, minimum volume is 4 mL.

1. Enzyme N 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. 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

2.2 Brain tumor dissociation protocol

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

▲ Remove necrotic tissue prior to determine the weight of the tumor sample.

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

1. 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.

2. 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.

3. Add the appropriate volume of Enzyme N, Buffer Y, and Enzyme A (refer to table in section 2.1) to the C Tube and mix gently.

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. If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_BTDK_1** and continue with step 14.
   If using the gentleMACS Octo Dissociator without heating function run program **h_tumor_02** and continue with step 6.

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

7. Incubate sample for 15 minutes at 37 °C under slow, continuous rotation using the MACSmix Tube Rotator.

8. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.

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. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.

13. Run gentleMACS Program **m_brain_01**.

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

15. Centrifuge briefly to collect the sample at the bottom of the tube.

16. Resuspend sample and apply it to a MACS® SmartStrainer (70 µm) placed on a 50 mL tube.

    ▲ **Note:** Moisten MACS SmartStrainer (70 µm) with buffer before use.

    ▲ **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.

17. 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.

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

19. 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 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.

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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. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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