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.
2. Skog, J. *et al.* (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat. Cell Biol. 10(12): 1470–1476.
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.**

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