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1. Description

This product is for research use only.

Components	<p>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</p>
Size	For 50 digestions of 2 mL.
Storage	<p>Upon arrival store Enzyme T of the Neural Tissue Dissociation Kit (T) and Enzyme P of the Neural Tissue Dissociation Kit (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 component refer to chapter 2.1.</p>

1.1 Principle of the Neural Dissociation Kits

Neural tissues from neonatal brain (animal age ≤ P7) 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 neural tissue is enzymatically digested using the kit components while the gentleMACS™ Dissociators are used for the mechanical dissociation steps. Cells should be processed immediately for downstream applications, such as cells separation, cell culture, cellular or molecular analyses.

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 from neonatal brain (≤ P7). 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

- Dissociations of neural tissues from neonatal brain (animal age ≤ P7) can be used directly for subsequent cell separations using MACS® Technology. Different neural cells can be isolated using MACS MicroBeads targeting specific neural antigens depending on the dissociation kit used: Neural Tissue Dissociation Kit (P), Neural Tissue Dissociation Kit (T), or Postnatal Neurons. For details refer to www.miltenyibiotec.com/130-092-628.
- *In vitro* cultivation of separated neural cells.
- Enumeration and phenotyping by flow cytometry or fluorescence microscopy.
- RNA or protein analysis, such as single-cell sequencing.

1.4 Reagent and instrument requirements

- Hanks' Balanced Salt Solution (HBSS) without Ca²⁺ and Mg²⁺ (Sigma-Aldrich # 55021C), in the following referred to as HBSS (w/o)
- HBSS with Ca²⁺ and Mg²⁺ (Sigma-Aldrich # 55037C), in the following referred to as HBSS (w)
- 50 mL tubes
- MACS SmartStrainers (70 µm) (# 130-098-462) for 50 mL tubes
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubation oven at 37 °C
- 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) MACS Neuro Medium (# 130-093-570)
- (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.

2. Protocol

2.1 Reagent preparation

▲ 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. When working with more than 400 mg, scale up all reagent volumes and total volumes accordingly. A maximum of 1600 mg mouse brain per C Tube can be processed.

1. Enzyme P and enzyme T are 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. Avoid repeated freeze-thaw-cycles.
2. Prepare enzyme mix 1 and enzyme mix 2 according to the table below.

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

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

▲ This protocol describes the dissociation of mouse brain tissue, though, in principle, it is transferable to other neural tissue types.

2.2.1 Dissociation using the gentleMACS™ Octo Dissociator with Heaters

1. Remove the mouse brain. Determine the weight of tissue in 1 mL of HBSS (w/o).
2. Transfer 1950 μL of enzyme mix 1 for up to 400 mg of tissue (refer to table in section 2.1) into a gentleMACS C Tube.
3. Transfer mouse brain into the C Tube containing enzyme mix 1.
4. Transfer 30 μL enzyme mix 2 (refer to table in section 2.1) 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 in the area of the rotator/stator.
6. Run the gentleMACS Program **37C_NTDK_1** and continue with step 14 of section 2.2.2.

2.2.2 Dissociation using the gentleMACS™ Dissociator or the gentleMACS Octo Dissociator

1. Remove the mouse brain. Determine the weight of tissue in 1 mL of HBSS (w/o).
2. Transfer 1950 μL of enzyme mix 1 for up to 400 mg of tissue (refer to table in section 2.1) into a gentleMACS C Tube and pre-heat at 37°C for 10–15 minutes.
3. Transfer mouse brain into the C Tube containing the pre-heated enzyme mix 1.
4. Tightly close C Tube and attach it upside down onto the sleeve of the dissociator.

▲ **Note:** It has to be ensured that the sample material is located in the area of the rotator/stator.
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. Transfer 30 μL enzyme mix 2 (refer to table in section 2.1) 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.
10. Incubate sample for 10 minutes at 37°C under slow, continuous rotation using the MACSmix™ Tube Rotator.
11. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.
12. Run the gentleMACS Program **m_brain_03**.
13. Incubate sample for 10 minutes at 37°C under slow, continuous rotation using the MACSmix Tube Rotator.
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 the cell suspension to a MACS SmartStrainer (70 μm) placed on a 50 mL tube.

▲ **Note:** Moisten MACS SmartStrainer 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 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 $>70\ \mu\text{m}$ 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 SmartStrainers (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 $300\times g$ 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 under slow, continuous rotation using the MACSmix Tube Rotator.

20. 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 excitotoxicity 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/130-092-628.

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.

Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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