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

This product is for research use only.

Components	**Neural Tissue Dissociation Kit (P)** 5 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) or **Neural Tissue Dissociation Kit (T)** 5 vials, containing: 1 vial of Enzyme T (lyophylized powder) 2×50 mL of Buffer X (sterile) 1.5 mL of Buffer Y (sterile) 1 vial of Enzyme A (lyophilized powder)
Size	For 50 digestions of 2 mL.
Storage	Upon arrival store Enzyme P of the Neural Tissue Dissociation Kit (P) aliquoted at –20 °C. Store all other components at +2 to +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 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

- gentleMACS Octo Dissociator with Heaters or gentleMACS Dissociator with MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at +37 °C.
- gentleMACS C Tubes (# 130-093-237)
- MACS SmartStrainers (70 µm) (# 130-098-462)
- 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)
- 50 mL reagent tubes
- Sterile water
- (Optional) MACS Neuro Medium (# 130-093-570)
- (Optional) MACS NeuroBrew®-21 (# 130-093-566)

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-001-846.16

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. **For NTDK (P):**
   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.

   For NTDK (T):
   Prepare Enzyme T by reconstitution of the lyophilized powder in the vial with 3 mL of Buffer X. Close the lid and invert the vial. Wait for 5–10 minutes. Do not pipette up and down. Prepare aliquots 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 the enzyme by inverting the vial immediately before taking out the required reaction volume.

2. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL of sterile water. Do not vortex. Prepare aliquots 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 the enzyme by inverting the vial immediately before taking out the required reaction volume

3. 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 60 µL	Buffer X 1890 µL	Buffer Y 20 µL	Enzyme A 10 µL

2.2 Neural tissue dissociation protocols

▲ For details on the use of gentleMACS Dissociators, refer to the respective user manual and www.miltenyibiotec.com/gentlemacs.

▲ 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

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.
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 reagent 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:** 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 SmartStrainers (70 µm) with an appropriate amount of HBSS (w), five times the enzyme solution volume. If necessary, split the sample.

140-001-846.16

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.

    ▲ **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. 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 for local Miltenyi Biotec Technical Support contact information.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.
THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.
All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.
The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.
CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

gentleMACS, MACS, MACSmix, the Miltenyi Biotec logo, and NeuroBrew are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.