Miltenyi Biotec

Neural Tissue Dissociation Kit – Postnatal Neurons

Order no. 130-094-802

Contents

1. Description
   - 1.1 Principle of the Neural Tissue Dissociation Kit – Postnatal Neurons
   - 1.2 Background information
   - 1.3 Applications
   - 1.4 Reagent and instrument requirements
2. Protocols
   - 2.1 Reagent and instrument preparation
   - 2.2 Neural tissue dissociation protocols
     - 2.2.1 Manual dissociation of retina
     - 2.2.2 Manual dissociation of brain tissue
     - 2.2.3 Automated dissociation of mouse brain tissue using the gentleMACS™ Dissociator or the gentleMACS™ Octo Dissociator
     - 2.2.4 Automated dissociation of mouse brain tissue using the gentleMACS™ Octo Dissociator with Heaters
3. References
4. Appendix: Tips & hints

1. Description

This product is for research use only.

Components	6 vials, containing: **2.5 mL of Enzyme P** **2×50 mL of Buffer Z (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 immediately store Enzyme P in aliquots at –20 °C. 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 Neural Tissue Dissociation Kit – Postnatal Neurons

Neural tissues can be dissociated to single-cell suspensions by enzymatic degradation of the extracellular adhesion proteins which maintain the structural integrity of tissues.

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 further mechanically dissociated and the suspension is applied to a MACS® SmartStrainer (70 µm). Cells should be processed immediately for downstream applications, such as cellular or molecular analyses or separations using MACS MicroBeads.

1.2 Background information

The Neural Tissue Dissociation Kit – Postnatal Neurons has been designed with regard to the special needs of sensitive neurons during dissociation. An optimal enzyme concentration ensures gentle enzymatic dissociation. The protocol was tested on rat retinas from 5–7 postnatal day (P5–7) old rats for subsequent isolation of retinal ganglion cells (RGC), which are the projection neurons of the eye[1-5] and on embryonic day 14 (E14)–P7 mouse brain tissue for the isolation of neurons. Brain tissue can be also dissociated automatically in a closed, sterile system using the gentleMACS™ Dissociators, which provide optimized programs to attain single-cell suspensions.

1.3 Applications

- Dissociation of human, mouse, or rat retinal tissue to single-cell suspensions for subsequent cell separation using MACS Technology, for example, isolation of retinal ganglion cells using the Retinal Ganglion Cell Isolation Kit, rat (# 130-096-209).
- Dissociation of mouse brain tissue for subsequent isolation of neurons using the Neuron Isolation Kit, mouse (# 130-098-752).
- Dissociation of neural tissue for *in vitro* cultivation or enumeration and phenotyping of neural cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- Dulbecco's phosphate-buffered saline (D-PBS) with calcium and magnesium.
- 50 mL tubes
- MACS SmartStrainers (70 µm) (# 130-098-462)
- 35 mm diameter sterile petri dish
- (Sterile) glass Pasteur pipettes
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator oven at 37 °C.
- (Optional) Beta-mercaptoethanol, 50 mM
- (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)
- (Optional) MACS Neuro Medium (# 130-093-570)
- (Optional) MACS NeuroBrew®-21 (# 130-093-566)
- (Optional) ART® 1000 REACH™ pipette tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

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Additional for manual dissociation:

- (Sterile) scalpel
- (Sterile) 5 mL pipette
- (Sterile) glass Pasteur pipettes
- 35 mm diameter sterile petri dish

2. Protocols

2.1 Reagent and instrument preparation

▲ For manual dissociation of neural tissue refer to section 2.2.1 or 2.2.2. For dissociation of neural tissue in combination with the gentleMACS™ Dissociators, please refer to section 2.2.3 or 2.2.4.

▲ For optimal results, volumes given below are for five retinas from P5–7 rats or a maximum of 400 mg of mouse brain tissue processed in 2 mL. When working with more than five retinas or more than 400 mg of mouse brain tissue, scale up all reagent volumes and total volumes accordingly.

1. (Optional for increased stability of enzymes) Add beta-mercaptoethanol to Buffer Z to a final concentration of 0.067 mM. For example, add 13.5 µL of 50 mM beta-mercaptoethanol to 10 mL of Buffer Z.
   ▲ **Note:** This solution will then be stable for 1 month at 4 °C.
2. 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. 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.
3. Prepare D-PBS supplemented with BSA, final concentration 0.5%; for example, 0.5 g BSA in 100 mL.
4. Prepare enzyme mix 1 and enzyme mix 2 according to the table below.

	Enzyme mix 1		Enzyme mix 2	
	Enzyme P	Buffer Z	Buffer Y	Enzyme A
Retina	10 µL	1950 µL	30 µL	15 µL
Brain tissue	50 µL	1910 µL	30 µL	15 µL

2.2 Neural tissue dissociation protocols

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

▲ Pre-warm Buffer Z to room temperature (19–25 °C).

▲ If using retina all steps must be performed at room temperature unless otherwise indicated.

▲ In case of subsequent gene expression profiling perform all steps at 4 °C.

▲ These protocols describe the dissociation of rat retinas or mouse brain tissue, though, in principle, they are transferable to other neural tissue types.

▲ All tubes, cell strainers, and pipette tips can be rinsed briefly with D-PBS to avoid stickinees.

▲ For the dissection of retinas a special protocol is available at www.miltenyibiotec.com/protocols.

▲ Operate the MACSmix™ Tube Rotator under slow, continuous rotation.

2.2.1 Manual dissociation of retina

1. Fire-polish a Pasteur pipette so that the opening has a diameter of approximately 0.8 mm and the edges are rounded. For details refer to 4. Appendix.
2. Dissect retinas from a minimum of five P5–7 rats in D-PBS according to the protocol "Dissection of retinas" available at www.miltenyibiotec.com/protocols.
3. Transfer tissue into a 15 mL tube using a 1 mL pipette tip. To facilitate pipetting cut approximately 2 mm off the end of the pipette tip.
4. Fill up with D-PBS to a final volume of 6 mL.
5. Let tissue settle. Remove supernatant carefully.
6. Add 1960 µL of enzyme mix 1 (refer to table above). Agitate gently to ensure that tissue is detached from the bottom of the tube.
7. Incubate in closed tubes for 15 minutes at 37 °C under slow, continuous rotation using a MACSmix Tube Rotator. Alternatively, incubate in a water bath, inverting the tube gently several times every 5 minutes to resuspend settled cells.
8. Add 30 µL of enzyme mix 2 (refer to table above).
9. Dissociate tissue using a wet 5 mL pipette by pipetting up and down the whole solution 10 times very carefully and slowly (without blow-out function). Avoid forming air bubbles.
10. Incubate for 10 minutes at 37 °C under slow, continuous rotation using a MACSmix Tube Rotator or in a water bath, agitating as described in step 7.
11. Add 15 µL of enzyme mix 2.
12. Dissociate tissue pieces using a wet 5 mL pipette by pipetting up and down 10 times very carefully and slowly. Dissociate tissue pieces further using a fire-polished Pasteur pipette that has an opening diameter of approximately 0.8 mm by pipetting approximately 2 mL up and down 35 times very carefully and slowly. Avoid forming air bubbles. Do not vortex.
13. Apply the cell suspension to a MACS® SmartStrainer (70 µm) placed on a 50 mL tube. Immediately add 10 mL of D-PBS supplemented with BSA.

    ▲ **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:** 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.
14. Centrifuge at 130×g for 10 minutes at room temperature. Remove supernatant carefully.
15. Resuspend cells carefully in D-PBS supplemented with BSA or medium to required volume by pipetting slowly up and down using a blue 1 mL pipette tip. Do not vortex the cells.
16. Count cells (dilution 1:100) using a Neubauer chamber or flow cytometer.
17. Cells should be processed immediately for further applications.

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2.2.2 Manual dissociation of brain tissue

1. Fire-polish three Pasteur pipettes so that decreasing tip diameters are achieved. For details refer to 4. Appendix.
2. Wash the brain in D-PBS and determine the weight of tissue to make sure that the 400 mg limit per digestion is not exceeded. Place the brain on the lid of a 35 mm diameter petri dish and cut brain into small pieced using a scalpel.
3. Transfer tissue into a 15 mL tube using a 1 mL pipette tip. To facilitate pipetting cut approximately 2 mm off the end of the pipette tip.
4. Fill up with D-PBS to a final volume of 6 mL.
5. Let tissue settle. Remove supernatant carefully.
6. Add 1960 µL of enzyme mix 1 (refer to table above). Agitate gently to ensure that tissue is detached from the bottom of the tube.
7. Incubate in closed tubes for 15 minutes at 37 °C under slow, continuous rotation using a MACSmix™ Tube Rotator. Alternatively, incubate in a water bath, inverting the tube gently several times every 5 minutes to resuspend settled cells.
8. Add 30 µL of enzyme mix 2 (refer to table above).
9. Dissociate tissue using a wide-tipped, fire-polished Pasteur pipette by pipetting up and down 10 times slowly. Avoid forming air bubbles.
10. Incubate for 10 minutes at 37 °C under slow, continuous rotation using a MACSmix Tube Rotator or in a water bath, agitating as described in step 7.
11. Add 15 µL of enzyme mix 2.
12. Dissociate tissue pieces using the other two fire-polished Pasteur pipettes with decreasing diameter. Pipette slowly up and down 10 times with each pipette, or as long as tissue pieces are still observable. Avoid forming air bubbles. Do not vortex.
13. Apply the cell suspension to a MACS® SmartStrainer (70 µm) placed on a 50 mL tube. Immediately add 10 mL of D-PBS supplemented with BSA.

    ▲ **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:** 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.
14. Centrifuge at 300×g for 10 minutes at room temperature. Remove supernatant carefully.
15. Resuspend cells carefully in D-PBS supplemented with BSA or medium to required volume by pipetting slowly up and down using a blue 1 mL pipette tip. Do not vortex the cells.
16. Count cells (dilution 1:100) using a Neubauer chamber or flow cytometer.
17. Cells should be processed immediately for further applications.

2.2.3 Automated dissociation of mouse brain tissue 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. Wash the brain in D-PBS and determine the weight of tissue to make sure that the 400 mg limit per digestion is not exceeded.
2. Transfer 1960 µL of enzyme mix 1 (refer to table above) into a gentleMACS C Tube.
3. Transfer mouse brain into the C Tube containing 1960 µL of the 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 in the area of the rotor/stator.
5. Run the gentleMACS Program **m_brain_01**.
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. Add 30 µL of the enzyme mix 2 (refer to table above). 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.
9. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.
10. Run the gentleMACS Program **m_brain_02**.
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. Add 15 µL of the enzyme mix 2 (refer to table above).
15. (Optional) 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. Immediately add 10 mL of D-PBS supplemented with BSA.

    ▲ **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.
17. Discard SmartStrainer and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate supernatant carefully.

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18. Resuspend cells carefully in D-PBS supplemented with BSA or medium to the required volume by pipetting slowly up and down. Do not vortex the cells.

20. Cells should be processed immediately for further applications.

2.2.4 Automated dissociation of mouse brain tissue 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.

1. Remove the mouse brain. Wash the brain in D-PBS and determine the weight of tissue to make sure that the 400 mg limit per digestion is not exceeded.
2. Transfer 1960 µL of enzyme mix 1 (refer to table above) into a gentleMACS C Tube.
3. Transfer mouse brain into the C Tube containing 1960 µL of the enzyme mix 1 per up to 400 mg of tissue.
4. Add 45 µL of the enzyme mix 2 (refer to table above). Invert gently to mix. Do not vortex.
5. Attach C Tube upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters.
6. Run the gentleMACS Program **37C_NTDK_1**.
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. Immediately add 10 mL of D-PBS supplemented with BSA.

   ▲ **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. Discard MACS SmartStrainer and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate supernatant carefully.
10. Resuspend cells carefully in D-PBS supplemented with BSA or medium to the required volume by pipetting slowly up and down. Do not vortex the cells.
11. Cells should be processed immediately for further applications.

3. References

1. Barres, B.A. *et al.* (1988) Immunological, morphological, and electrophysiological variation among retinal ganglion cells purified by panning. Neuron 1: 791–803.
2. Harada, T. *et al.* (2007) Molecular regulation of visual system development: more than meets the eye. Genes & Dev. 21: 367–378.
3. Goritz, C. *et al.* (2007) Glia-induced neuronal differentiation by transcriptional regulation. Glia 55: 1108–1122.
4. Kyungsuk Park, K. *et al.* (2008) Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science 322: 963–966.

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
Retina: Rotate the pipette quickly in the flame to fire polish it for a few seconds. Fast rotation of pipettes is facilitated by applying a rubber sucker. Too much heat may fuse the opening. The edges should be rounded. The opening should not be smaller than approximately 0.8 mm so that the cells are not forced through with too much pressure.

Brain tissue: 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.

Yield of 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. During the working steps at 37 °C the MACSmix™ Tube Rotator is convenient for this purpose. Keep pipetting until the single-cell suspension looks homogeneous. Then apply suspension to the 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 30 µL of enzyme mix 2 (20 µL of Buffer Y and 10 µL of Enzyme A) per 2 mL, incubate for 5–10 minutes at 37 °C, centrifuge, and wash again.

Single-cell suspension contains many dead cells
Make sure that the openings of the Pasteur pipettes are not too small. Pipette more slowly and do not vortex the cells.

Staining does not work (epitope is lost)
Enzyme P can be diluted 1:5 or 1:10.
Alternatively, incubate the dissociated cells in medium for two hours at 37 °C under rotation in MACSmix Tube Rotator to allow re-expression.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols.