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1. Description

1.1 Background information

Single-cell suspensions are a prerequisite for many experiments, for example to achieve the highest possible purity and recovery during cell separations with MACS® Technology. The gentleMACS™ Dissociator provides optimized programs to attain single-cell suspensions from various tissues, for example, mouse neural tissue. In combination with C Tubes, the gentleMACS Dissociator allows the automated tissue dissociation in a closed system, enabling sterile sample handling. A single sample or two samples can be processed in parallel.

1.2 Reagent and instrument requirements

- gentleMACS Dissociator
- gentleMACS C Tubes (# 130-093-237)
- MACSmix™ Tube Rotator (# 130-090-753)
- Pre-Separation Filters (# 130-041-407)
- Neural Tissue Dissociation Kit (P) (# 130-092-628) or Neural Tissue Dissociation Kit (T) (# 130-093-231)
- (Optional) ART 1000 REACH pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.
- Hanks' Balanced Salt Solution (HBSS) without Ca²⁺ and Mg²⁺ (Sigma-Aldrich #H4891), in the following referred to as HBSS (w/o)
- HBSS with Ca²⁺ and Mg²⁺ (Sigma-Aldrich #H1387), in the following referred to as HBSS (w)
- Beta-mercaptoethanol (e.g. Sigma, # 63689)

2. Protocol for the dissociation of mouse neural tissue

2.1 Reagent preparation

The components of the Neural Tissue Dissociation Kits (NTDK) are listed in table 1.

	Solution 1 [mL]	Solution 2 [mL]	Solution 3 [mL]	Solution 4 [mg]	Storage Buffer [mL]
NTDK (P)	2.5	2×50	1.5	5–15	1
NTDK (T)	10	2×50	1.5	5–15	1

Table 1: Components of the Neural Tissue Dissociation Kits (NTDK).

1. Add beta-mercaptoethanol to Solution 2 to a final concentration of 0.067 mM. For example, add 13.5 µL of 50 mM beta-mercaptoethanol to 10 mL of Solution 2.
▲ **Note:** This solution will then be stable for 1 month at 4 °C.
2. Resuspend the lyophilized powder in the vial labeled Solution 4 with 1 mL of Storage Buffer for Solution 4. Do **not** vortex. This solution should then be sterile filtered in the case of cell culture applications, aliquoted and stored at –20 °C for later use.
3. Prepare 1950 µL enzyme mix 1 for up to 400 mg tissue according to table 2 and vortex. Pipette enzyme mix 1 into gentleMACS™ C Tube and pre-heat the mixture at 37 °C for 10–15 minutes before use. Proceed to 2.2 Sample preparation.

	Enzyme mix 1		Enzyme mix 2	
	Solution 1	Solution 2	Solution 3	Solution 4
NTDK (P)	50 µL	1900 µL	20 µL	10 µL
NTDK (T)	200 µL	1750 µL	20 µL	10 µL

Table 2: Preparation of the enzyme mixes.

2.2 Sample preparation

- ▲ For details on the use of the gentleMACS Dissociator, refer to the gentleMACS Dissociator user manual.
- ▲ Volumes given below are for the dissociation of up to 400 mg mouse brain. 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. The total volume should not exceed 10 mL.
- ▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.
- ▲ The MACSmix™ Tube Rotator is used at permanent run at a speed of approximal 4 rpm.

1. Remove the mouse brain. Determine the weight of tissue in 1 mL of HBSS (w/o).
2. Transfer mouse brain into the gentleMACS C Tube containing 1950 μ L of the pre-heated enzyme mix 1 per up to 400 mg of tissue (see 2.1 Reagent preparation, step 3).
3. 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.
4. Switch on the gentleMACS Dissociator and choose the gentleMACS Program **m_brain_01**.
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. Attach C Tube 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.
9. Choose and run the gentleMACS Program **m_brain_02**.
10. After termination of the program **m_brain_02**, detach C Tube from the gentleMACS Dissociator.
11. Prepare 30 μ L enzyme mix 2 per up to 400 mg tissue, according to table 2.
12. 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 μ L pipette tips.
13. Incubate sample for 10 minutes at 37 °C under slow, continuous rotation using the MACSmix Tube Rotator.
14. Attach C Tube 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.
15. Choose and run the gentleMACS Program **m_brain_03**.
16. After termination of the program, detach C Tube from the gentleMACS Dissociator.
17. Incubate sample for 10 minutes at 37 °C under slow, continuous rotation using the MACSmix Tube Rotator.
18. (Optional) Centrifuge briefly to collect the sample at the bottom of the tube.
19. Resuspend sample and apply the cell suspension to a Pre-Separation Filter placed on a 15 mL tube.
 ▲ **Note:** When working with more than 400 mg mouse brain use a 50 mL tube and an appropriate cell strainer.
 ▲ **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 μ L pipette tips.
 ▲ **Note:** Cells with a diameter > 30 μ m, such as Purkinje cells or motoneurons, may be lost. To obtain these cells within the flow through, use a cell strainer with an appropriate mesh size.
20. Wash Pre-Separation Filter with 10 mL of HBSS (w).
 ▲ **Note:** When working with more than 400 mg mouse brain wash cell strainer with an appropriate amount of HBSS (w) up to 30 mL.
21. Discard Pre-Separation Filter and centrifuge cell suspension at 300 \times g for 10 minutes at room temperature. Aspirate supernatant completely.
22. (Optional) Resuspend cell suspension in 10 mL HBSS (w) and centrifuge at 300 \times g for 10 minutes at room temperature. Aspirate supernatant completely.
23. 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 μ L of enzyme mix 2 per mL of cell suspension, mix gently and incubate for a minimum of 5 min at 37 °C using the MACSmix Tube Rotator. Repeat steps 22 and 23.

All gentleMACS Protocols are available at www.miltenyibiotec.com.

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