Miltenyi Biotec

Dissociation of inflamed neural tissue using the Multi Tissue Dissociation Kit 1

Contents

1. Description

1.1 Background information

Chronic inflammation is typically associated with neurodegenerative diseases like multiple sclerosis (MS), Parkinson's disease, Alzheimer's disease, Huntington's disease, or ALS. The experimental autoimmune encephalomyelitis (EAE) is an animal model of brain inflammation as an inflammatory demyelinating disease of the central nervous system (CNS) and the most commonly used experimental model for MS. During neuroinflammation immune cells such as macrophages, T cells, and B cells infiltrate CNS.

This dissociation protocol has been developed with special attention on the preservation of those epitopes (refer to table below) to allow subsequent analysis and isolation of immune cells from inflamed neural tissue. In case of subsequent detection or isolation of neural cells, e.g., neurons, astrocytes, oligodendrocytes, or microglia, the use of the Adult Brain Dissociation Kit, mouse and rat is recommended.

Inflammed neural tissue is dissociated into single-cell suspensions using the Multi Tissue Dissociation Kit 1 in combination with the gentleMACS™ Octo Dissociator with Heaters. The extracellular matrix is enzymatically digested using the kit components, while the gentleMACS Octo Dissociator with Heaters is used for the mechanical dissociation steps during the on-instrument enzyme incubation. After the dissociation, the myelin and cell debris is removed using the Debris Removal Solution followed by a subsequent removal of erythrocytes using the Red Blood Cell Lysis Solution.

Preserved surface epitope	Recommended antibody clone
CD3ε	REA641
CD4	REA604
CD8a	REA601
CD11b	REA592
CD11c	REA754
CD19	REA749
CD20	REA294
CD25*	REA568
CD27	REA499
CD45	REA737
CD45R (220)	REA755
CD49b	REA541
CD68	REA835
F4/80	REA126
Ly-6G	REA526
Ly-6C	REA796
NK1.1	PK136

* Epitope-sensitive, can result in reduced detection level.

1.2 Reagent and instrument requirements

- Multi Tissue Dissociation Kit 1 (# 130-110-201)
- gentleMACS Octo Dissociator with Heaters (# 130-096-427) and gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- Serum-free RPMI 1640 or DMEM
- Dulbecco's phopshate-buffered saline (D-PBS) with calcium, magnesium, glucose, and pyruvate. Keep buffer cold (2–8 °C).
- PB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2 and 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with PBS. Keep buffer cold (2–8 °C). Always use freshly prepared buffer. Do **not use** autoMACS® Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.

  ▲ **Note:** BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS).
- Debris Removal Solution (# 130-109-398)
- Red Blood Cells Lysis Solution (10×) (# 130-094-183)
- MACS SmartStrainers (70 µm) (# 130-098-462)
- Double-distilled water (ddH_2O)
- 35 mm diameter sterile petri dish
- 5 or 15 mL and 50 mL reagent tubes

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Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 877 591 1060
macs@miltenyibiotec.com

- Centrifuge with a swinging bucket rotor
- (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

▲ For subsequent cell separation it is recommended to dissociate at least 800 mg of neural tissue.

▲ Volumes given below are for one adult mouse brain (400–500 mg) or up to 500 mg of mouse spinal cord in 2500 µL enzyme mix. When working with less than 500 mg, use the same volumes as indicated. When working with more than 500 mg, determine the weight and scale up all reagent volumes and total volumes accordingly. A maximum of 500 mg neural tissue per C Tube can be processed.

▲ The use of centrifuges with a swinging bucket rotor rather than centrifuges with a fixed angle rotor is highly recommended.

1. Prepare Enzyme D of the Multi Tissue Dissociation Kit 1 by reconstitution of the lyophilized powder in each vial with 3 mL of serum-free RPMI 1640 or DMEM. Close the vial and wait for at least 5 minutes while inverting every minute. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. For cell culture experiments subsequent to tissue dissociation, Enzyme D should be sterile filtered prior to aliquoting.
   Store aliquots at –20 °C. This solution is stable for 6 months.
2. Prepare Enzyme R of the Multi Tissue Dissociation Kit 1 by reconstitution of the lyophilized powder in the vial with 2.7 mL serum-free RPMI 1640 or DMEM. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months.
   ▲ **Note:** Make sure to thoroughly mix this suspension immediately before withdrawing the required reaction volume!
3. Prepare Enzyme A of the Multi Tissue Dissociation Kit 1 by reconstitution of the lyophilized powder in the vial with 1 mL of Buffer A supplied with the of the Multi Tissue Dissociation Kit 1. Do not vortex. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles.
   Store aliquots at –20 °C. This solution is stable for 6 months.
4. Prepare 1× Red Blood Cell Lysis Solution by diluting the Red Blood Cell Lysis Solution (10×) 1:10 with ddH_2O, for example, dilute 0.1 mL of cold Red Blood Cell Lysis Solution (10×) with 0.9 mL cold ddH_2O. Store the prepared 1× Red Blood Cell Lysis Solution at 2–8 °C. Discard unused solution at the end of the day.
   ▲ **Note:** Do not use deionized water for dilution!
5. Prepare enzyme mix according to the table below.

Enzyme mix			
Enzyme D 100 µL	Enzyme R 50 µL	Enzyme A 12.5 µL	D-PBS 2338 µL

2.2 Dissociation protocol

▲ For details on the use of the gentleMACS™ Octo Dissociator with Heaters, refer to the user manual.

▲ A maximum of one adult mouse brain (400–500 mg) or up to 500 mg of mouse spinal cord in 2.5 mL enzyme mix can be processed in one C Tube.

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

▲ Moisten MACS® SmartStrainer (70 µm) with 5 mL D-PBS before use.

1. Remove the mouse brain or spinal cord. Wash the brain in cold D-PBS.
2. Prepare the appropriate volume of enzyme mix (refer to table in section 2.1) and transfer it into a gentleMACS C Tube.
3. Place the neural tissue into a petri dish and cut it into slices using a scalpel. Cut the mouse brain into approximately 8 sagittal slices. Cut the spinal cord into pieces of approximately 5 mm length.
4. Transfer the tissue pieces into the C Tube containing 2500 µL of enzyme mix.
5. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters.
6. Run the gentleMACS Program **37C_Multi_F**.
7. After termination of the program, detach C Tube from the gentleMACS Octo Dissociator with Heaters.
8. (Optional) Centrifuge briefly to collect the sample at the bottom of the tube.
9. Resuspend sample and apply it to a moistened 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 one adult mouse brain.
   ▲ **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.
10. Apply 10 mL of cold D-PBS onto the MACS SmartStrainer (70 µm).
11. Discard MACS SmartStrainer (70 µm) and centrifuge cell suspension at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.
12. Proceed to 2.3 for debris and red blood cell removal.

2.3 Debris and red blood cell removal

▲ Volumes given below are for the cell suspension from up to 1 g neural tissue as starting material. When working with higher tissue quantities, scale up all reagent volumes and total volumes accordingly.

▲ A maximum of cell suspension from up to 1 g neural tissue can be processed in one 15 mL reagent tube or cell suspension from one mouse spinal cord in one 5 mL tube.

▲ Always use pre-cooled buffers and solutions (4 °C).

	Reagent tube volume	Debris Removal Solution	D-PBS	Overlay (D-PBS)
1 spinal cord	5 mL	450 µL	1550 µL	2 mL
1 brain (400–500 mg)	15 mL	900 µL	3100 µL	4 mL
2 brains (800–1000 mg)	15 mL	1800 µL	6200 µL	4 mL

1. Resuspend cell pellet carefully with appropriate volume of cold D-PBS according to the table above and transfer cell suspension to the appropriate reagent tube. Do not vortex.
2. Add appropriate volume of cold Debris Removal Solution according to the table above.
3. Mix well.
4. Overlay very gently with appropriate volume of cold D-PBS according to the table above.

   ▲ **Note:** Pipette very slowly to ensure that the D-PBS phase overlays the cell suspension and phases are not mixed.
5. Centrifuge at 4 °C and 3000×g for 10 minutes with full acceleration and full brake.

   ▲ **Note:** Some centrifuges may give suboptimal centrifugation results. In this case the acceleration and brake can be reduced.
6. Three phases are formed. Aspirate the two top phases completely and discard them.
7. Fill up with cold D-PBS.
8. Gently invert the tube three times. Do not vortex!
9. Centrifuge at 4 °C and 1000×g for 10 minutes with full acceleration and full brake. Aspirate supernatant completely.

   ▲ **Note:** When working with perfused brain or perfused spinal cord, red blood cell removal is optional and you can continue with step 14.
10. Resuspend cell pellet carefully in 1 mL of cold 1× Red Blood Cell Removal Solution. Do not vortex.
11. Incubate for 10 minutes in the refrigerator (2–8 °C).
12. Add 10 mL of cold PB buffer.
13. Centrifuge at 4 °C and 300×g for 10 minutes. Aspirate supernatant completely.
14. Resuspend cells carefully in the appropriate buffer or medium by pipetting slowly up and down. Do not vortex.
15. Cells should be processed immediately for further applications, e.g., flow cytometric analysis or isolation of immune cells.

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

Warranty