Miltenyi Biotec

Adult Brain Dissociation Kit
mouse and rat

Order no. 130-107-677

Contents

1. Description

This product is for research use only.

Components	7 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)** **5 mL of Red Blood Cell Removal Solution (10×) (sterile)** **45 mL of Debris Removal Solution (sterile)**
Size	For 50 digestions. The specified number of digestions is valid when digesting rodent neural tissue from 20 mg up to 500 mg following the protocol in chapter 2.2.
Storage	Upon arrival immediately store Enzyme P in aliquots at –20 °C. Store all other components at +2 to +8 °C upon arrival. Reconstitute Enzyme A before the date indicated on the vial label. For information about reconstitution and storage after reconstitution of the lyophilized components refer to chapter 2.1.

1.1 Principle of the Adult Brain Dissociation Kit

Neural tissue from adult mice or rats 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. After dissociation, the Debris Removal Solution is used for the removal of debris followed by an subsequent removal of erythrocytes using the Red Blood Cell Removal Solution.
Cells should be processed immediately for downstream applications, such as cell separation, cell culture, cellular or molecular analyses.

1.2 Background information

The Adult Brain Dissociation Kit has been developed for the gentle, rapid, and efficient generation of single-cell suspensions from adult neural tissue considering the special needs of sensitive neural cells from adult mice or rats during dissociation. Adult brain, distinct brain regions (e.g. cerebral hemispheres, hippocampus, or subventricular zone), or spinal cord can be used as starting material. The optimized gentleMACS Protocol ensures gentle mechanical dissociation and a high yield of viable cells, after removal of debris and red blood cells. The gentleMACS Program, the debris removal protocol, and the erythrocyte removal protocol differ from small amounts of neural tissue (20–100 mg) to higher amounts of neural tissue (> 100 mg) to ensure the optimum result for all applications.
The single-cell suspension can be analyzed *in vitro* for phenotype distributions, and other functional, genetic, or proteomic studies can be performed. Furthermore, dissociated cells can be subsequently isolated or cultured using MACS® Technology. In case of subsequent cultivation it is recommended to use at least 800 mg of neural tissue as starting material for dissociation.

1.3 Applications

- Dissociation of neural tissue from adult mice or rats, including whole brain, brain regions (such as hippocampus, cerebral hemispheres, or subventricular zone etc.) and spinal cord, into single-cell suspensions free of debris and red blood cells, which can be used directly for subsequent:
  - cell separations using MACS Technology, for example, isolation of astrocytes using the Anti-ACSA-2 MicroBead Kit, mouse (# 130-097-678), isolation of neurons using the Neuron Isolation Kit, mouse (# 130-115-390), isolation of oligodendrocytes using the Anti-O4 MicroBeads, human, mouse, rat (# 130-094-543), or isolation of microglia using the CD11b (Microglia) MicroBeads, human and mouse (# 130-093-634) or CD11b/c (Microglia) MicroBeads, rat (# 130-105-634),
  - *in vitro* cultivation of separated neural cells,
    ▲ **Note:** In case of subsequent cultivation it is recommended to use at least 800 mg of neural tissue as starting material for dissociation
  - enumeration and phenotyping by flow cytometry or fluorescence microscopy, or
  - RNA or protein analysis, such as single-cell sequencing.

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1.4 Reagent and instrument requirements

- gentleMACS Octo Dissociator with Heaters
- gentleMACS C Tubes (# 130-093-237)
- MACS SmartStrainer (70 µm) (# 130-098-462)
- (Optional) MACS SmartStrainer (100 µm) (# 130-110-916)
- Dulbecco's phosphate-buffered saline (D-PBS) with calcium, magnesium, glucose, and pyruvate. Keep buffer cold (+2 to +8 °C).
- PB buffer: Prepare a solution containing D-PBS, pH 7.2, and 0.5% bovine serum albumin (BSA) by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with D-PBS. Keep buffer cold (+2 to +8 °C). Degas buffer before use, as air bubbles could block the column.
  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 mouse or rat serum albumin, mouse or rat serum, or fetal bovine serum (FBS).
- Sterile, deionized water for reconstitution of Enzyme A
- Purified water (e.g., double-distilled water (ddH_2O)) for preparation of 1× Red Blood Cell Lysis Solution.

  ▲ **Note:** Do not dilute with deionized water.
- 35 mm diameter sterile petri dish
- 15 mL and 50 mL reagent tubes
- Centrifuge with a swinging bucket rotor
- 5 mL reagent tubes (e.g. Sigma Aldrich #Z688223-200EA or Corning #352058)
- (Optional) MACS Neuro Medium (# 130-093-570) and MACS NeuroBrew®-21 (# 130-093-566) for subsequent cell culture.

2. Protocol

2.1 Reagent and instrument preparation

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

▲ Volumes given below are for one adult mouse brain (max. 500 mg) in 1980 µL enzyme mix. When working with less than 500 mg, use the same volumes as indicated. When working with neural tissue from adult rats or distinct brain regions, 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.

▲ A swinging bucket rotor is recommended for centrifugation, e.g., Heraeus® Multifuge 4KR by Thermo Fisher® Scientific.

1. 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 sterile, deionized water. 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	
Enzyme P	Buffer Z	Buffer Y	Enzyme A
50 µL	1900 µL	20 µL	10 µL

2.2 Adult brain dissociation protocol

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

▲ 20 mg up to 500 mg of rodent neural tissue in 2 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.

1. Remove the rodent neural tissue. Wash neural tissue in cold D-PBS.
2. Prepare the appropriate volume of enzyme mix 1 (refer to table in section 2.1) and transfer it into a gentleMACS C Tube.
3. Cut larger tissue (e.g., whole brain or spinal cord) into approximately 8 sagittal slices or 0.5 cm pieces using a scalpel. In case of smaller tissue, e.g., hippocampus continue directly with step 4.
4. Transfer the tissue pieces into the C Tube containing 1950 µL of enzyme mix 1.
5. Transfer 30 µL of enzyme mix 2 into the C Tube.
6. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters.
7. Run the appropriate gentleMACS Program:
   20–100 mg: **37C_ABDK_02**
   >100 mg: **37C_ABDK_01**.
8. After termination of the program, detach C Tube from the gentleMACS Octo Dissociator with Heaters.
9. Centrifuge briefly to collect the sample at the bottom of the tube.
10. Resuspend sample and apply it 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 one adult mouse brain.

    ▲ **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, e.g., MACS SmartStrainer (100 µm).
11. Add 10 mL of cold D-PBS to the C Tube, close C Tube, shake gently and apply the D-PBS onto the MACS SmartStrainer (70 µm).
12. Discard MACS SmartStrainer (70 µm) and centrifuge cell suspension at 300×g for 10 minutes at +4 °C. Aspirate supernatant completely.
13. Proceed to 2.3 for debris removal.

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2.3 Debris removal

▲ Volumes given below are for the cell suspension from 20 mg up to a maximum of 1 g neural tissue (~2 adult mouse brain) as starting material. When working with higher tissue quantities, scale up all reagent volumes and total volumes accordingly.

▲ Cell suspensions from a maximum of 1 g neural tissue (~2 adult mouse brain) can be processed in one 15 mL reagent tube or cell suspensions from 20–100 mg in one 5 mL tube.

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

	D-PBS	Debris Removal Solution	Overlay (D-PBS)	Reagent tube
20–100 mg	1550 µL	450 µL	2 mL	5 mL
400–500 mg (~1 brain)	3100 µL	900 µL	4 mL	15 mL
800–1000 mg (~2 brains)	6200 µL	1800 µL	4 mL	15 mL

1. Resuspend cell pellet carefully with the appropriate volume of cold D-PBS according to the table above and transfer cell suspension to an appropriate reagent tube. Do not vortex.
2. Add appropriate volume of cold Debris Removal Solution.
3. Mix well.
4. Overlay very gently with an appropriate amount 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:** If centrifuges give suboptimal centrifugation, 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.
10. Proceed to 2.4 for red blood cell removal.

2.4 Red blood cell removal

2.4.1 Preparation of 1× Red Blood Cell Removal Solution

1. Dilute the Red Blood Cell Removal Solution (10×) 1:10 with purified water, for example, dilute 1 mL of cold Red Blood Cell Removal Solution (10×) with 9 mL cold ddH_2O.

   ▲ **Note:** Do not use deionized water for dilution!
2. Store the prepared 1× Red Blood Cell Removal Solution at +2 to +8 °C. Discard unused solution at the end of the day.

2.4.2 Red blood cell removal

▲ Volumes given below are for cell suspensions from 20 mg up to a maximum of 1 g neural tissue (~2 adult mouse brain) as starting material. When working with higher amounts of starting material, scale up all volumes accordingly.

1. Resuspend cell pellet from up to two adult mouse brains carefully in the appropriate amount of cold 1× Red Blood Cell Removal Solution:

   20–100 mg: 0.5 mL

   100–1000 mg: 1 mL. Do not vortex.
2. Incubate for 10 minutes in the refrigerator (+2 to +8 °C).
3. Add an appropriate amount of cold PB buffer:

   20–100 mg: 5 mL

   100–1000 mg: 10 mL.
4. Centrifuge at +4 °C and 300×g for 10 minutes. Aspirate supernatant completely.
5. Resuspend cells carefully in the appropriate buffer or medium by pipetting slowly up and down. Do not vortex.
6. Cells should be processed immediately for further applications.

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.

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