Miltenyi Biotec

Myelin Removal Beads II
human, mouse, rat

4 mL	130-096-733
2×4 mL	130-096-433

Contents

1. Description

Components	**4 mL Myelin Removal Beads II, human, mouse, rat** or **2× 4 mL Myelin Removal Beads II, human, mouse, rat**
Capacity	**Up to 200 separations** or **up to 400 separations**
Product format	Myelin Removal Beads II are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS® Separation

First, the myelin debris is magnetically labeled with MicroBeads, the Myelin Removal Beads II. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled myelin is retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of myelin.

1.2 Background information

Myelin, a specialized membrane, ensheathes and insulates axons in the peripheral and central nervous system. In mice and rats, myelination begins around birth in the spinal cord and is completed in the brain during the first postnatal month.[1] In humans, myelin formation starts during the second half of fetal life in the spinal cord, peaks during the first year postnatally and can continue until twenty years of age.[2]

When myelin containing neural tissue is dissociated, large quantities of myelin debris are generated. Magnetic cell isolation and immunostaining of dissociated cells are considerably impaired by myelin particles.[3,4] So far, sucrose solution[5,6] has been used for the elimination of myelin but leads to unspecific cell loss.
Myelin Removal Beads II allow for the specific removal of myelin debris from single-cell suspensions during sample preparation, leading to a higher purity and recovery of target cells, and thereby highly improving subsequent cell isolation and antibody staining. Myelin removal is therefore recommended prior to cell sorting or antibody staining.

1.3 Applications

- Removal of myelin debris from single-cell suspensions from human, mouse, or rat (rodents older than postnatal day 7–10, depending on the species or strain) during sample preparation for significant improvement of cell separation efficiency and immunostainings.

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 0.5% bovine serum albumin (BSA) by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with PBS. Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** BSA can be replaced by other proteins such as appropriate serum albumin, appropriate serum, or fetal bovine serum (FBS).

- MACS Columns and MACS Separators: Myelin can be depleted with the use of LS Columns. Depletion can also be performed by using the autoMACS® Pro or the autoMACS Separator.

  ▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

- (Optional) Neural Tissue Dissociation Kit (P) (# 130-092-628), Neural Tissue Dissociation Kit (T) (# 130-093-231), Brain Tumor Dissociation Kit (P) (# 130-095-942), or Brain Tumor Dissociation Kit (T) (# 130-095-939) for the preparation of single-cell suspensions from neural tissues.
- (Optional) gentleMACS™ Dissociator (# 130-093-235).
- (Optional) Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.

2. Protocol

2.1 Sample preparation

For the preparation of single-cell suspensions from neural tissues refer to the data sheet of the Neural Tissue Dissociation Kit (P), the Neural Tissue Dissociation Kit (T), the Brain Tumor Dissociation Kit (P), or the Brain Tumor Dissociation Kit (T), which can be used in combination with the gentleMACS Dissociator.

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2.2 Magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ The recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

▲ The older the mouse the more myelin is present. Therefore the volumes of buffer and Myelin Removal Beads II depend on the age and should be adjusted according to the following table.

Mouse	<2 weeks old	2–3 weeks old	>3 weeks old
Weight	300 mg	400 mg	500 mg
Volume of buffer per whole mouse brain	180 µL	1080 µL	1800 µL
Volume of Myelin Removal Beads II per whole mouse brain	20 µL	120 µL	200 µL
Number of LS Columns required	1 (for up to 2 brains)	2	3
autoMACS Pro Separator / autoMACS Separator: Number of autoMACS Columns required	1 (for up to 2 brains)	1	1

The table above refers to CD1 mice. If brain derived from Balb/c or C57/BL6 mice is used, weight may be different and should be determined.

For example, when using three P18 mouse brains (postnatal day 18), add 3240 µL of buffer (3×1080 µL) and 360 µL Myelin Removal Beads II (3×120 µL) to the cell pellet. After the washing step add 6000 µL of buffer (6×1000 µL) to the cell pellet because you need 6 LS Columns and apply 1000 µL of your suspension to each LS Column.

For dissected tissue pieces instead of whole mouse brain:
Weigh the tissue pieces and calculate their weight in relation to a whole brain. Divide the volumes for buffer and Myelin Removal Beads II by that factor.
For example, a cerebellum of a P18 mouse weighs approximately 100 mg. Therefore, divide volumes of buffer and Myelin Removal Beads II by a factor of 4. When using only the cerebella from the three P18 mouse brains in the example above, use 810 µL of buffer (3240 µL:4) and 90 µL Myelin Removal Beads II (360 µL:4). After the washing step, add 2000 µL of buffer to the cell pellet because you need 2 LS Columns and apply 1000 µL of your suspension to each LS Column.

For rat brain tissue:
Weigh the rat brain tissue and calculate their weight in relation to a whole mouse brain. Multiply the volumes for buffer and Myelin Removal Beads II by that factor.

For example, a brain of a P18 rat weighs approximately 800 mg. Therefore, multiply volumes of buffer and Myelin Removal Beads II by a factor of 2. Use 2160 µL of buffer (2×1080 µL) and 240 µL Myelin Removal Beads II (2×120 µL). After the washing step, add 4000 µL of buffer to the cell pellet because you need 4 LS Columns and apply 1000 µL of your suspension to each LS Column.

For human tissue (any age):
For a single-cell suspension from a human sample of any age use 1800 µL of buffer, 200 µL of Myelin Removal Beads II, and 3 LS Columns per 500 mg of tissue.

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Add volume of buffer as according to table above.
3. Add volume of Myelin Removal Beads II as according to table above.
4. Mix well. Do not vortex. Incubate for 15 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 10× the labeling volume of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Add 1000 µL of buffer per each LS Column and 2000 µL of buffer per each autoMACS Column to the cell pellet.

   ▲ **Note**: For example, if you need 3 LS Columns according to the table, add 3000 µL of buffer to the cell pellet (3×1000 µL) and apply 1000 µL to each LS Column.
7. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose the appropriate number of LS Columns according to the number of total cells. For details refer to tables in section 2.2.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation. Pass cells through 70 µm nylon mesh (Pre-Separation Filters, 70 µm # 130-095-823) or appropriate mesh size depending on target cells to remove cell clumps which may clog the column. Moisten filter with buffer before use.

Depletion with LS Columns

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to LS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

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Magnetic separation with the autoMACS® Pro Separator or the autoMACS® Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator or the autoMACS Separator.

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of ≥10 °C.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

▲ Do not use the Neural Tissue Dissociation Kit (T) or the Brain Tumor Dissociation Kit (T) in combination with the autoMACS Pro Separator or the autoMACS Separator.

Magnetic separation with the autoMACS® Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

   Depletion: DepleteS
   Collect negative fraction in row B of the tube rack.

Magnetic separation with the autoMACS® Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

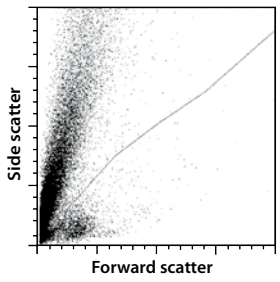
   Depletion: DepleteS
   Collect negative fraction from outlet port neg1.

3. Example of myelin depletion using Myelin Removal Beads II

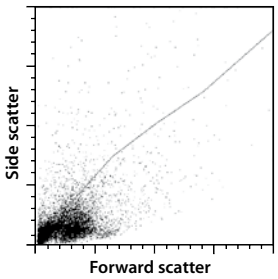
Myelin debris is abundant in single-cell suspensions

Mouse brains were dissociated using the Neural Tissue Dissociation Kit (P) (# 130-092-628). Flow cytometrical analysis of the resulting single-cell suspensions shows the distribution of cells and myelin debris (A). Single-cell suspensions from P28 (postnatal day 28) mouse brains consist of large amounts of myelin membrane fragments, other cellular debris and only 3% cells. Myelin Removal Beads II used with LS Columns and MidiMACS Separator efficiently remove myelin debris (B).

A) Before myelin removal



B) After myelin removal



4. References

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All protocols and data sheets are available at **www.miltenyibiotec.com**.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

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