Myelin Isolation Beads
human, mouse, rat

200 µL	130-104-262
4 mL	130-104-257
2×4 mL	130-104-253

Contents

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.

1. Description

This product is for research use only.

Components	**200 µL Myelin Isolation Beads, human, mouse, rat – small size** or **4 mL Myelin Isolation Beads, human, mouse, rat** or **2×4 mL Myelin Isolation Beads, human, mouse, rat:** MicroBeads conjugated to monoclonal anti-mouse myelin antibodies (mouse IgM).
Capacity	**Up to 10 separations** or **up to 200 separations** or **up to 400 separations**
Product format	Myelin Isolation Beads 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, myelin debris in a single-cell suspension are magnetically labeled with Myelin Isolation Beads. 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 neural cells run through; this cell fraction is thus depleted of myelin. After removing the column from the magnetic field, the magnetically retained myelin can be eluted as the positively selected fraction and it can be used for further experiments or flow cytometric analysis.

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. 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.
The Myelin Isolation Beads allow the enrichment of myelin debris from single-cell suspension for quantitative determination of the myelin amount, which is of particular interest for de- and remyelination studies.

1.3 Application

- Positive selection of myelin debris from single-cell suspension from human, mouse, or rat tissue (rodents older than postnatal day 14), or from cell culture.
- Enrichment of myelin debris for subsequent quantitative analysis using flow cytometer, preferably the MACSQuant® Analyzer.

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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS).

- MACS Columns and MACS Separators: Myelin can be isolated using LS Columns and the QuadroMACS™ Separator. Positive selection can also be performed by using the autoMACS Pro Separator or the MultiMACS™ Cell24 Separator.

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

- Neural Tissue Dissociation Kit (P) (# 130-092-628)
- Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps.
- (Optional) For quantification of myelin debris: Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Labeling Check Reagent, PE (# 130-119-813). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

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- (Optional) gentleMACS™ Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937)
- (Optional) MACSQuant® Analyzer 10 (# 130-096-343)

2. Protocol

2.1 Sample preparation

For preparation of single-cell suspensions from neural tissues the use of the Neural Tissue Dissociation Kit (P) (# 130-092-628) is highly recommended to guarantee reliable results, especially in case of subsequent quantitative analysis. For details refer to the respective data sheet. The kit can be used in combination with the gentleMACS Dissociator (# 130-092-235) or gentleMACS Octo Dissociator (# 130-095-937).

Refer to the protocols section at www.miltenyibiotec.com/protocols for details.

▲ For the application note "Gating strategy for quantification of myelin debris" refer to **www.miltenyibiotec.com/myelin**.

2.2 Magnetic labeling

▲ Cells can be labeled with MACS® MicroBeads using the autolabeling function of the autoMACS Pro Separator. For more information refer to section 2.4.

▲ 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. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

Mouse tissue

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

For mouse brain	<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 Isolation Beads 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: 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 Isolation Beads (3×120 µL) to the cell pellet. After incubation fill up to 6000 µL with buffer (6×1000 µL) 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 Isolation Beads by that factor.

For example, a cerebellum of a P18 mouse weighs approximately 100 mg. Therefore, divide volumes of buffer and Myelin Isolation Beads 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 Isolation Beads (360 µL:4). After incubation fill with buffer up to 2000 µL because you need 2 LS Columns and apply 1000 µL of your suspension to each LS Column.

Rat tissue

Weigh the rat brain tissue pieces and calculate their weight in relation to a whole mouse brain. Multiply the volumes for buffer and Myelin Isolation Beads by that factor. For example, a brain of a P18 rat weighs approximately 800 mg. Therefore, multiply volumes of buffer and Myelin Isolation Beads by a factor of 2. Use 2160 µL of buffer (2×1080 µL) and 240 µL Myelin Isolation Beads (2×120 µL). After incubation fill up to 4000 µL of buffer because you need 4 LS Columns and apply 1000 µL of your suspension to each LS Column.

Human tissue

Weigh the human brain tissue pieces and calculate their weight in relation to a whole mouse brain. Multiply the volumes for buffer and Myelin Isolation Beads by that factor. For example, for 500 mg human tissue of any age use 1800 µL of buffer, 200 µL of Myelin Isolation Beads, and 3 LS Columns.

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 Isolation Beads as according to table above.
4. Mix well. Do not vortex. Incubate for 15 minutes in the refrigerator (2–8 °C).
5. (Optional) For flow cytometric analysis add the same volume of Labeling Check Reagent as of the Myelin Isolation Beads after 5 minutes of incubation. Mix well. Incubate for the remaining 10 minutes.
6. Fill with buffer to an appropriate volume, so that 1000 µL of cell suspension can be applied on one LS Column.

   ▲ **Note:** For example, if you need 3 LS Columns according to the table, fill with buffer up to3000 µL and apply 1000 µL to each LS Column.
7. Proceed to magnetic separation (2.3 or 2.4).

2.3 Magnetic separation

▲ Choose the appropriate number of LS MACS Columns and MACS Separator according to the number of total cells. For details refer to the table 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 labeling. 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.

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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.
5. For the isolation of the magnetically labeled myelin remove the column from the separator and place the column on a suitable collection tube.
6. Pipette 3 mL of buffer onto the column and immediately flush out the magnetically labeled myelin by firmly pushing the plunger into the column.

Magnetic separation with the MultiMACS™ Cell24 Separator

Refer to the MultiMACS Cell Separator user manual for instructions on how to use the MultiMACS Cell24 Separator.

2.4 Cell separation with the autoMACS® Pro Separator

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

▲ All buffer temperatures should be ≥10 °C.

▲ For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.

▲ Place tubes in the following Chill Rack positions:
position A = sample, position B = negative fraction,
position C = positive fraction.

2.4.1 Fully automated cell labeling and separation

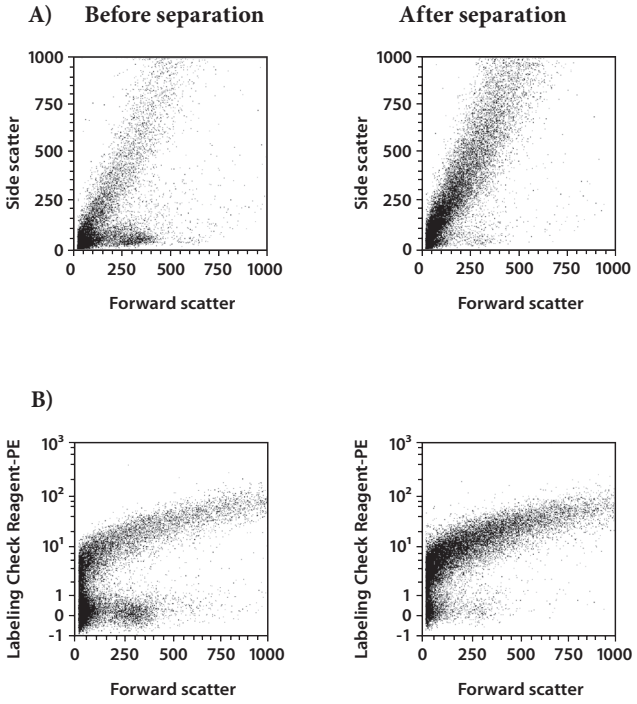
1. Switch on the instrument for automatic initialization.
2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS Pro Separator. Place the reagent into the appropriate position on the reagent rack.
3. Place sample and collection tubes into the Chill Rack.
4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation, and wash protocols will be selected automatically).
5. Enter sample volume into the **Volume** submenu. Press **Enter**.
6. Select **Run**.

2.4.2 Magnetic separation using manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample and collection tubes into the Chill Rack.
4. For a standard separation choose the following program:
   Positive selection: Possels
   Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled myelin.

3. Example of a separation using Myelin Isolation Beads

Myelin was isolated from P35 (postnatal day 35) CD1 mouse brain tissue using the Neural Tissue Dissociation Kit (P), the gentleMACS™ Octo Dissociator, Myelin Isolation Beads, a MidiMACS™ Separator, and LS Columns. Myelin was fluorescently stained using the Labeling Check Reagent-PE and analyzed using the MACSQuant® Analyzer. Analysis of the myelin isolation was performed either using the scatter properties (A) or the fluorescent staining of the myelin (B).



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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