Miltenyi Biotec

Anti-O4 MicroBeads
human, mouse, rat

1 mL	130-094-543
50 µL	130-096-670

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	**1 mL Anti-O4 MicroBeads, human, mouse, rat** or **50 µL Anti-O4 MicroBeads, human, mouse, rat – small size:** MicroBeads conjugated to monoclonal anti-mouse O4 antibodies (mouse IgM).
Capacity	1 mL for 1×10^9 total cells, up to 100 separations or 50 µL for 5×10^7 total cells, up to 5 separations.
Product format	Anti-O4 MicroBeads 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.

Cross-reactivity: The Anti-O4 antibody has been tested to react with mouse and rat cells. Cross-reactivity for human cells has been successfully tested by flow cytometry. The Anti-O4 antibody has been reported to react with chicken cells.

1.1 Principle of the MACS® Separation

First, the $O4^+$ cells are magnetically labeled with Anti-O4 MicroBeads. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled $O4^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $O4^+$ cells. After removing the column from the magnetic field, the magnetically retained $O4^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the $O4^+$ cells can be separated over a second column.

1.2 Background information

The O4 antigen, a sulfatide, which belongs to the class of glycosphingolipids, is a marker for oligodendrocytes. During oligodendrocyte development, O4 expression begins on late oligodendrocyte progenitors that are A2B5-positive. While A2B5 expression disappears, O4 continues to be expressed. As the cells differentiate, they synthesize galactocerebroside, myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoproetein (MOG), and O1. In the peripheral nervous sytem, O4 expression is found in Schwann cells. The isolation of $O4^+$ cells leads to highest purities if P3–P7 (postnatal day 3–7) rodents are used. Dissociated mouse brain tissue derived from P3–P7 CD1 mice contains approximately 5–10% $O4^+$ cells.

1.3 Applications

- Positive selection or depletion of cells expressing O4 antigen.
- Isolation of $O4^+$ oligodendrocytes from dissociated mouse or rat brain tissue (derived from P3–P7 rodents).

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: $O4^+$ cells can be enriched by using MS or LS Columns or depleted with the use of LD Columns. Positive selection or depletion can also be performed by using the autoMACS Pro Separator or the MultiMACS™ Cell24 Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^7	MiniMACS, OctoMACS
LS	2×10^7	4×10^7	MidiMACS, QuadroMACS
Depletion			
LD	1.5×10^7	3×10^7	MidiMACS, QuadroMACS
Positive selection or depletion			
autoMACS	5×10^7	10^8	autoMACS Pro
Multi-24	2×10^7	4×10^7	MultiMACS Cell24

▲ **Note:** For depletion with LD Columns, prepare a single-cell suspension using the Neural Tissue Dissociation Kit (P) (# 130-092-628)

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- Neural Tissue Dissociation Kit (P) (# 130-092-628) or Neural Tissue Dissociation Kit (T) (# 130-093-231) for the generation of single-cell suspensions of neural cells from mouse brain tissue.

  ▲ **Note:** The Neural Tissue Dissociation Kit (P) is recommended in combination with Anti-O4 MicroBeads.

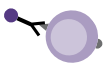
- Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.
- (Optional) Fluorochrome-conjugated Anti-O4 antibodies for flow cytometric analysis, e.g., Anti-O4-PE (# 130-095-887), Anti-O4-APC (# 130-095-891), or Anti-O4-Biotin (# 130-095-895). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (#130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-O4-Biotin.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) or FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) MACS NeuroMedia (# 130-093-570) and MACS NeuroBrew-21 (# 130-093-566) for the cultivation of oligodendrocytes.
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)

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) (# 130-092-628) or of the Neural Tissue Dissociation Kit (T) (# 130-093-231), which can both be used in combination with the gentleMACS Dissociator (#130-092-235).

For details refer to the protocols section at www.miltenyibiotec.com/protocols.



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.

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. For best performance it is recommended to use at least 5×10^6 cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ 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) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ 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.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Add 90 µL of buffer per 10^7 total cells to the cell pellet.

   ▲ **Note:** For animals older than P7, add 97.5 µL of buffer per 10^7 total cells to the cell pellet.

   ▲ **Note:** If FcR Blocking Reagent, mouse is being used, add 80 µL of buffer and 10 µL of FcR Blocking Reagent, mouse per 10^7 nucleated cells. Mix well. Do not vortex. Incubate for 10 minutes in the refrigerator (2–8 °C).
   If FcR Blocking Reagent, human is being used, add 70 µL of buffer and 20 µL of FcR Blocking Reagent, human per 10^7 nucleated cells. Mix well. Do not vortex. Incubate for 10 minutes in the refrigerator (2-8°C).

4. Add 10 µL of Anti-O4 MicroBeads per 10^7 total cells.

   ▲ **Note:** For animals older than P7, add 2.5 µL of Anti-O4-MicroBeads per 10^7 total cells.

5. Mix well. Do not vortex. Incubate for 15 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend up to 10^7 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

8. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $O4^+$ cells. For details refer to the table in section 1.4.

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

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:

   MS: 500 µL LS: 3 mL

3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   MS: 3×500 µL LS: 3×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.

5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column.

Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

MS: 1 mL LS: 5 mL

7. (Optional) To increase the purity of $O4^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

▲ **Note:** Elution of the cells from the column after the separation should be performed with cell culture medium if cells are to be taken directly into culture, otherwise elute with buffer as before.

▲ **Note:** Keep handling time of cells in PBS/BSA buffer to a minimum.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.
2. Prepare column by rinsing with 2 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 flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

Magnetic separation with the MultiMACS™ Cell24 Separator

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

Magnetic separation with the autoMACS® Pro Separator

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

▲ Buffers used for operating the autoMACS Pro 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.

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 one of the following programs:

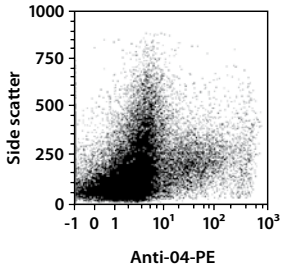
   Positive selection: Posseld2
   Collect positive fraction in row C of the tube rack.

   Depletion: Depl05
   Collect negative fraction in row B of the tube rack.
   Collect negative fraction from outlet port neg1.

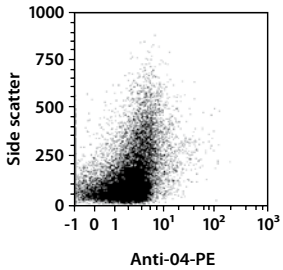
3. Example of a separation using Anti-O4 MicroBeads

$O4^+$ cells were isolated from day 6 postnatal mouse brain tissue using the Neural Tissue Dissociation Kit (P), the gentleMACS Dissociator, FcR Blocking Reagent, Anti-O4 MicroBeads, an OctoMACS™ Separator, and two MS Columns. Cells were fluorescently stained with Anti-O4-PE (# 130-095-887) in 1 mL before cells were applied onto the column and analyzed using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

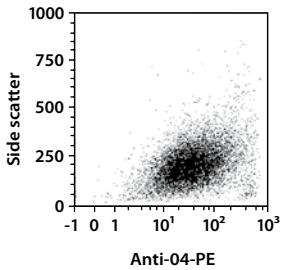
Before separation



$O4^-$ cells



$O4^+$ cells



4. References

1. Zhang, S. C. (2001) Defining glial cells during CNS development. Nat. Rev. Neurosci. 2: 840–843.
2. Sommer, I. and Schachner, M. (1981) Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. Dev. Biol. 83: 311–327.

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

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