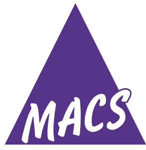

Anti-A2B5 MicroBeads

human, mouse, rat

Order no. 130-093-388

Contents

1. Description
    1.1 Principle of MACS® Separation
    1.2 Background information
    1.3 Applications
    1.4 Reagent and instrument requirements
2. Protocol
    2.1 Sample preparation
    2.2 Magnetic labeling
    2.3 Magnetic separation
3. Example of a separation using Anti-A2B5 MicroBeads
4. References

1. Description

Components	2 mL Anti-A2B5 MicroBeads: MicroBeads conjugated to monoclonal anti-mouse A2B5 antibodies (isotype: mouse IgM).
Capacity	For 1×10^9 total cells, up to 100 separations.
Product format	Anti-A2B5 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.

1.1 Principle of MACS® Separation

First, the A2B5⁺ cells are magnetically labeled with Anti-A2B5 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 A2B5⁺ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of A2B5⁺ cells. After removing the column from the magnetic field, the magnetically retained A2B5⁺ cells can be eluted as the positively selected cell fraction.

1.2 Background information

Anti-A2B5 MicroBeads have been developed for the isolation of A2B5⁺ cells. The Anti-A2B5 MicroBeads recognize the c-series ganglioside antigen A2B5. Glial-restricted progenitors from embryonic to adult human, mouse, and rat tissue have been immunomagnetically isolated according to the expression of A2B5.[1-10]

A2B5 is predominantly expressed in embryonic and neonatal neural tissue. In adult mammalian brain A2B5 expression is restricted mainly to areas that retain neurogenic potential such as the subventricular zone (SVZ).

Thus, A2B5 is considered as a marker for immature glial-committed precursors that are permanently generated in the SVZ. Glial-restricted precursor cells are defined as cells that give rise to glial cell types, such as astrocytes and oligodendrocytes.

Ganglioside GT3 and its O-acetylated derivative are recognized by A2B5, and both antigens are down-regulated as the cells differentiate into mature oligodendrocytes.

1.3 Applications

- Positive selection of cells expressing A2B5.
- Positive selection of neuronal progenitor cells, for example, from mouse SVZ tissue in conjunction with prior depletion of A2B5⁺ glial progenitor cells.[4]

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS) pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (2−8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: A2B5⁺ cells can be enriched by using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS or the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
Positive selection			
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

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

- Neural Tissue Dissociation Kit (T) (# 130-093-231) or Neural Tissue Dissociation Kit (P) (# 130-092-628) for the generation of single-cell suspensions of neural cells from mouse and rat neural tissue.
- Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- Cell culture medium, e.g., DMEM/F12 (Sigma-Aldrich D6421)

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- FcR Blocking Reagent, mouse (# 130-092-575) or human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the labeling and discrimination of dead cells by flow cytometry.

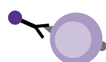
2. Protocol

2.1 Sample preparation

When working with tissues, prepare a single-cell suspension by a standard preparation method. For details see section General Protocols in the User Manuals or visit www.miltenyibiotec.com/protocols.

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

For the preparation of cells from neural tissue refer to the data sheet of the Neural Tissue Dissociation Kit (T) (# 130-093-231) or the Neural Tissue Dissociation Kit (P) (# 130-092-628).



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. 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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column.

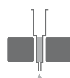
▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. When working with **mouse** cells, resuspend cell pellet in 70 µL of buffer per 10^7 total cells.

   When working with **human** cells, resuspend cell pellet in 60 µL of buffer per 10^7 total cells.
4. (Optional) When working with **mouse** cells, add 10 µL of FcR Blocking Reagent, mouse per 10^7 total cells.

   (Optional) When working with **human** cells, add 20 µL of FcR Blocking Reagent, human per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2−8 °C).
6. Add 20 µL of Anti-A2B5 MicroBeads per 10^7 total cells. Mix well and incubate for 15 minutes in the refrigerator (2−8 °C).
7. Wash cells by adding 1−2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. (Optional) Add staining antibodies according to the manufacturer's recommendations.
9. Wash cells by adding 1−2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
10. Resuspend up to 10^8 cells in 500 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
11. 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 A2B5⁺ cells. For details see table in section 1.4.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette an 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 purity of A2B5⁺ cells, the eluted fraction can be directly enriched over a second 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 second 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 times of cells in PBS/EDTA/BSA buffer to a minimum. Cells must only be stored in cell cuture medium after enrichment over the columns in order to preserve cell viability.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.

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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.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details see CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way stopcock of the assembled column. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total effluent; this is the unlabeled cell fraction.

Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

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

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

▲ Buffers used for operating the autoMACS Separator or 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.

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 one of the following programs:

   Positive selection: "Possel"
   Collect positive fraction from outlet port pos1.

   Depletion: "Depl05"
   Collect negative fraction from outlet port neg1.

Magnetic separation with the autoMACS™ Pro Separator

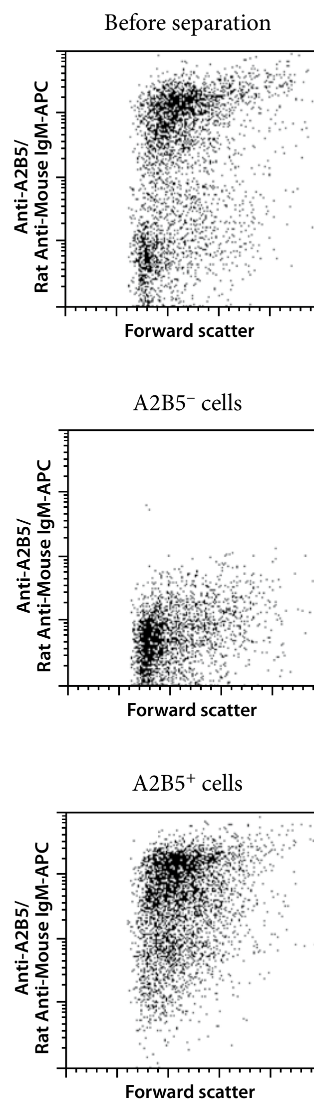
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

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

   Depletion: "Depl05"
   Collect negative fraction in row B of the tube rack

3. Example of a separation using Anti-A2B5 MicroBeads

Separation of a single-cell suspension derived from day 1 postnatal mouse whole-brain tissue using the Neural Tissue Dissociation Kit (P), Anti-A2B5 MicroBeads, a MiniMACS™ Separator and an MS Column. Cells were fluorescently stained with an APC-conjugated rat anti-mouse IgM antibody and analyzed by flow cytometry. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.



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4. References

1. Windrem, M. S. *et al.* (2004) Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. Nat. Med. 10: 93–97. Erratum in: Nat. Med. (2004) 10: 209.
2. Ruffini, F. *et al.* (2004) Distinctive properties of human adult brain-derived myelin progenitor cells. Am. J. Pathol. 165: 2167–2175.
3. Larsen, P. H. *et al.* (2006) Myelin formation during development of the CNS is delayed in matrix metalloproteinase-9 and -12 null mice. J. Neurosci. 26: 2207–2214.
4. Seidenfaden, R. *et al.* (2006) Glial conversion of SVZ-derived committed neuronal precursors after ectopic grafting into the adult brain. Mol. Cell Neurosci. 32: 187–198.
5. Larsen, P. H. and Yong, V. W. (2004) The expression of matrix metalloproteinase-12 by oligodendrocytes regulates their maturation and morphological differentiation. J. Neurosci. 24: 7597–7603.
6. Liu, A. *et al.* (2007) The glial or neuronal fate choice of oligodendrocyte progenitors is modulated by their ability to acquire an epigenetic memory. J. Neurosci. 27: 7339–7343.
7. Strahmann, F. G. *et al.* (2007) Identification of two novel glial-restricted cell populations in the embryonic telencephalon arising from unique origins. BMC Dev. Biol. 7: 33.
8. Larsen, P. H. *et al.* (2003) Matrix metalloproteinase-9 facilitates remyelination in part by processing the inhibitory NG2 proteoglycan. J. Neurosci. 23: 11127–11135.
9. Dietrich, J. *et al.* (2002) Characterization of A2B5+ glial precursor cells from cryopreserved human fetal brain progenitor cells. Glia 40: 65–77.
10. Dubois, C. *et al.* (1990) Monoclonal antibody A2B5, which detects cell surface antigens, binds to ganglioside GT3 (II3 (NeuAc)3LacCer) and to its 9-O-acetylated derivative. J. Biol. Chem. 265: 2797–2803.

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.

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