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## 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.

<b>Components</b>	1 mL Anti-A2B5 MicroBeads, human, mouse, rat or 100 µL Anti-A2B5 MicroBeads, human, mouse, rat – small size: MicroBeads conjugated to monoclonal anti-mouse A2B5 antibodies (isotype: mouse IgM).
<b>Capacity</b>	1 mL for $5 \times 10^8$ total cells, up to 50 separations or 100 µL for $5 \times 10^7$ total cells, up to 5 separations.
<b>Product format</b>	Anti-A2B5 MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	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<sup>+</sup> 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<sup>+</sup> cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of A2B5<sup>+</sup> cells. After removing the column from the magnetic field, the magnetically retained A2B5<sup>+</sup> 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<sup>+</sup> 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.<sup>1–10</sup>

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<sup>+</sup> glial progenitor cells.<sup>4</sup>

## 1.4 Reagent and instrument requirements

- **Buffer:** Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 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). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.

- **MACS Columns and MACS Separators:** AN2<sup>+</sup> 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
<b>Positive selection</b>			
MS	10 <sup>7</sup>	2×10 <sup>7</sup>	MiniMACS, OctoMACS
LS	2×10 <sup>7</sup>	4×10 <sup>7</sup>	MidiMACS, QuadroMACS
<b>Depletion</b>			
LD	1.5×10 <sup>7</sup>	3×10 <sup>7</sup>	MidiMACS, QuadroMACS
<b>Positive selection or depletion</b>			
autoMACS	5×10 <sup>7</sup>	10 <sup>8</sup>	autoMACS Pro
Multi-24	2×10 <sup>7</sup>	4×10 <sup>7</sup>	MultiMACS Cell24

- 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.
- MACS Smart Strainers (30  $\mu$ m) (# 130-098-458) or Pre-Separation Filters, 30  $\mu$ m (# 130-041-407) to remove cell clumps.
- MACS Neuro Medium (# 130-093-570) and MACS NeuroBrew-21 (# 130-093-566) for cultivation.
- FcR Blocking Reagent, mouse (# 130-092-575) or human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., IgM-APC. For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

## 2. Protocol

### 2.1 Sample preparation

For the preparation of single-cell suspensions from neural tissues refer to data sheet of Neural Tissue Dissociation Kit (T) (# 130-093-231) or the Neural Tissue Dissociation Kit (P) (# 130-092-628), which can be used in combination with the gentleMACS Dissociators.

For details refer to the protocols section at [www.miltenyibiotec.com/protocols](http://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. 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  $2 \times 10^7$  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  $\mu$ m nylon mesh (MACS Smart Strainers (30  $\mu$ m) (# 130-098-458) or Pre-Separation Filters, 30  $\mu$ m, # 130-041-407) to remove cell clumps which may clog the column.

▲ 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 $\times$ g for 10 minutes. Aspirate supernatant completely.
3. When working with **mouse** cells, resuspend cell pellet in 70  $\mu$ L of buffer per  $10^7$  total cells.

When working with **human** cells, resuspend cell pellet in 60  $\mu$ L of buffer per  $10^7$  total cells.

4. (Optional) When working with **mouse** cells, add 10  $\mu$ L of FcR Blocking Reagent, mouse per  $10^7$  total cells.

(Optional) When working with **human** cells, add 20  $\mu$ L 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  $\mu$ L 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 $\times$ g for 10 minutes. Aspirate supernatant completely.
8. (Optional) Add staining antibodies, e.g., 10  $\mu$ L of IgM-APC, and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
9. Wash cells by adding 1–2 mL of buffer per  $10^7$  cells and centrifuge at 300 $\times$ g for 10 minutes. Aspirate supernatant completely.
10. Resuspend up to  $10^8$  cells in 500  $\mu$ L 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<sup>+</sup> cells. For details refer to the 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 refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:  
MS: 500  $\mu$ L      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 $\times$ 500  $\mu$ L      LS: 3 $\times$ 3 mL

▲ **Note:** Perform washing steps by adding buffer aliquots as soon as 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 A2B5<sup>+</sup> 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 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 culture 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 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 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 refer to the 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 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  $\geq 10^\circ\text{C}$ .

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

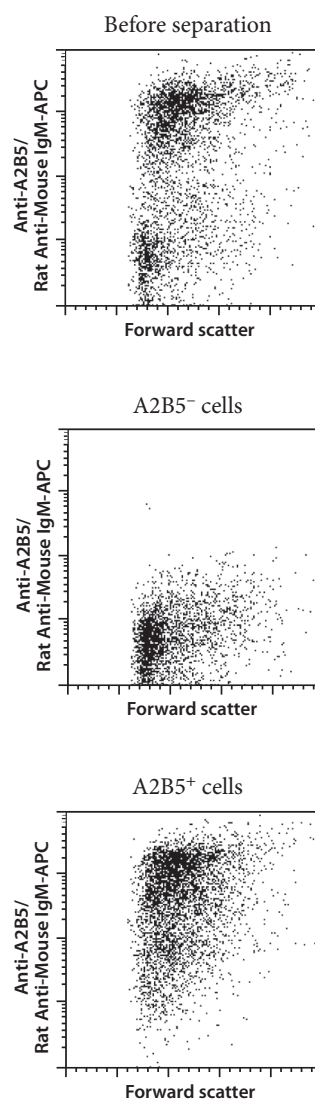
Collect positive fraction in row C of the tube rack.

#### 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 propidium iodide fluorescence.



## 4. References

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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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