Miltenyi Biotec

Anti-A2B5 MicroBead Kit
human and mouse

Order no. 130-097-864

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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.

Components	**1 mL Anti-A2B5-APC, human and mouse:** monoclonal anti-A2B5 antibody conjugated to APC (clone: 105-HB29; isotype: mouse IgM). **2 mL Anti-APC MicroBeads:** MicroBeads conjugated to monoclonal anti-APC antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	All reagents 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 pluripotent stem cell-derived A2B5$^+$ cells are indirectly magnetically labeled with Anti-A2B5-APC and Anti-APC 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

The Anti-A2B5 MicroBead Kit has been developed for the separation of human and mouse pluripotent stem cell-derived glial precursor cells based on the expression of the A2B5 antigen. Ganglioside GT3 and its O-acetylated derivative are the principal A2B5-reactive gangliosides.[1] *In vitro* differentiation of human and mouse pluripotent stem cells under defined conditions revealed that oligodendrocyte precursor cells express the surface marker A2B5.[2,3] Magnetic enrichment of *in vitro* generated glial precursors will facilite access to a highly pure A2B5-positive cell population and may even shorten established differentiation protocols.

1.3 Applications

- Positive selection of A2B5$^+$ cells derived from mouse or human pluripotent stem cells.

1.4 Reagent and instrument requirements

- DPBS buffer: Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+}.
- PB buffer: Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+} and 0.5% bovine serum albumin (BSA). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.
- MACS Columns and MACS Separators: For optimal purity and recovery the use of an LS Column is strongly recommended. Positive selection can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
LS	10^8	2×10^9	MidiMACS, QuadroMACS
autoMACS	2×10^8	4×10^9	

- (Optional) Embryoid Body Dissociation Kit, mouse and human (# 130-096-348).
- (Optional) Trypsin solution: 0.05% trypsin, 2 mM EDTA.
- (Optional) Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.
- (Optional) 5 mL serological pipette.
- (Optional) MACSmix™ Tube Rotator (# 130-090-753).

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2. Protocol

2.1 Sample preparation

Below mentioned differentiation protocols have been used to optimize enrichment of glial precursors derived from pluripotent stem cells using the Anti-A2B5 MicroBead Kit.

Preparation of mouse cells
Pluripotent stem cells were differentiated as embryoid bodies for 4–5 days in suspension with 1×10^5 cells/mL in DMEM and 10% fetal bovine serum. The Embryoid Body Dissociation Kit (# 130-096-348) was used to generate single-cell suspensions prior to antibody labeling.

Preparation of human cells
Differentiation of human pluripotent stem cells followed the protocols published by Chambers *et al.*[4] and Zhou *et al.*[5]
In brief, 2×10^6 pluripotent stem cells were plated on Matrigel®-coated 10 cm dishes in DMEM/F12 medium supplemented with 20% serum replacement, dorsomorphin (1 µM) and thiazovivin (2 µM).

After 10 days of differentiation cells were harvested as followed:

1. Remove differentiation medium and wash culture plates twice with DPBS buffer.
2. Detach cells by addition of 2 mL of trypsin solution (0.05% trypsin, 2 mM EDTA) per 10 cm culture dish for 5 minutes at 37 °C.
3. Stop enzymatic reaction by addition of soybean trypsin inhibitor.
4. Dissociate to single-cell suspension by pipetting up and down using a 5 mL serological pipette.
5. Pass cells through 70 µm nylon mesh (Pre-Separation Filters, # 130-095-823) to remove cell clumps which may clog the column. Moisten filter with buffer or culture medium before use.

   ▲ **Note:** Prolonged trypsin treatment of cells might reduce A2B5 antibody labeling efficiency due to trypsin sensitivity of the A2B5 epitope.



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 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 5 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 100 µL of PB buffer per 10^7 total cells.
4. Add 10 µL of Anti-A2B5-APC per 10^7 total cells
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** (Optional) The MACSmix Tube Rotator can be used for continuous mixing of larger volumes. Operate MACSmix Tube Rotator on permanent run at a speed of approximately 4 rpm.
6. Wash cells by adding 1–2 mL of PB buffer per 10^7 cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 80 µL of PB buffer per 10^7 total cells.
8. Add 20 µL of Anti-APC MicroBeads per 10^7 total cells.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).

   ▲ **Note:** (Optional) The MACSmix Tube Rotator can be used for continuous mixing of larger volumes. Operate MACSmix Tube Rotator on permanent run at a speed of approximately 4 rpm.
10. Wash cells by adding 1–2 mL of PB buffer per 10^7 cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^7 cells in 500 µL of PB buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).



2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator. 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 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 LS Column by rinsing with 3 mL of PB buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×3 mL of PB buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   ▲ **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 5 mL of PB buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

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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.

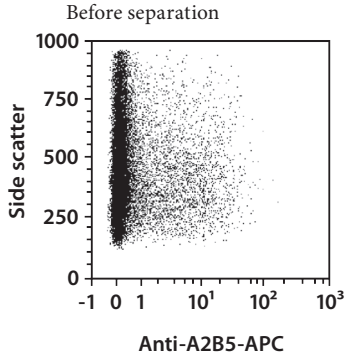
▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

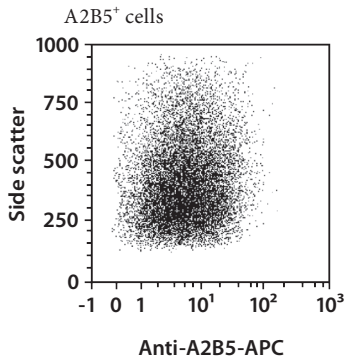
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

   Positive selection of human cells: posseld
   Positive selection of mouse cells: possel
   Collect positive fraction in row C of the tube rack.

3. Example of a separation using the Anti-A2B5 MicroBead Kit

A2B5$^+$ cells were isolated from human pluripotent stem cell–derived glial precursor cells using the Anti-A2B5 MicroBead Kit, an LS Column, and a MidiMACS™ Separator. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.





4. References

1. 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.
2. Sundberg, M. *et al.* (2011) A xeno-free culturing protocol for pluripotent stem cell-derived oligodendrocyte precursor cell production. Regen Med. 6: 449–460.
3. Tokumoto, Y. *et al.* (2010) Comparison of efficiency of terminal differentiation of oligodendrocytes from induced pluripotent stem cells versus embryonic stem cells *in vitro*. J. Biosci Bioeng 109: 622–628.
4. Chambers, S. M. *et al.* (2009) Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat. Biotechnol. 27: 275–280.
5. Zhou, J. *et al.* (2010) High-efficiency induction of neural conversion in human ESCs and human induced pluripotent stem cells with a single chemical inhibitor of transforming growth factor beta superfamily receptors. Stem Cells 28: 1741–1750.

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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