Miltenyi Biotec

Anti-AN2 MicroBeads
human and mouse

1 mL	130-097-170
100 µL	130-097-171

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-AN2 MicroBeads, human and mouse** or **100 µL Anti-AN2 MicroBeads, human and mouse – small size:** MicroBeads conjugated to monoclonal anti-mouse AN2 antibodies (isotype: rat IgG1).
Capacity	1 mL for 1×10^9 total cells, up to 100 separations or 100 µL for 1×10^8 total cells, up to 10 separations.
Product format	Anti-AN2 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 the MACS® Separation

First, the AN2$^+$ cells are magnetically labeled with Anti-AN2 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 AN2$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of AN2$^+$ cells. After removing the column from the magnetic field, the magnetically retained AN2$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

NG2 is a chondroitin sulfate glycoprotein that is expressed by glial progenitor cells in the developing and adult central nervous system (CNS) and by immature Schwann cells. The mouse homologue of the rat NG2 and human MCSP protein is called AN2[1].
NG2/AN2$^+$ glia have been identified as a fourth major glial cell type in the mammalian CNS that is distinct from other cell types.[2] NG2/AN2$^+$ cells are precursors of oligodendrocytes and some protoplasmic astrocytes in the grey matter[3]. NG2/AN2 expression is down regulated before the cells undergo terminal differentiation. However, a population of NG2/AN2$^+$ cells remain in the white and grey matter after oligodendrocytes are generated.[4–6]
NG2/AN2$^+$ glia participate in neuron-glial circuits by receiving presynaptic input from neurons and responding to neurotransmitters released by synapses[7]. Many highly migratory neural tumors including melanomas express NG2/AN2. AN2$^+$ cells were successfully isolated from P1–P7 (postnatal day 1–7) dissociated mouse brain tissue derived from CD1 mice containing approximately 5–12% AN2$^+$ cells.

1.3 Applications

- Positive selection or depletion of cells expressing AN2 antigen.
- Isolation of AN2$^+$ cells from dissociated mouse brain tissue, derived from mice younger than eight days (<P8).

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^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: AN2$^+$ 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

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- (Optional) Fluorochrome-conjugated Labeling Check Reagents to stain labeled cells for flow cytometric analysis, e.g., Labeling Check Reagent-PE (# 130-095-228). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

  ▲ **Note:** The use of Anti-AN2 antibodies, clone 1E6.4, is not recommended for analysis of cells that are labeled with Anti-AN2 MicroBeads.

- Re-expression and cultivation medium: Prepare a medium by diluting MACS NeuroBrew-21 (# 130-093-566) 1:50 and L-glutamine (200 mM) 1:100 in MACS Neuro Medium (# 130-093-570).
- MACSmix™ Tube Rotator (# 130-090-753)
- The Neural Tissue Dissociation Kit (P) (# 130-092-628) is recommended for the generation of single-cell suspensions of neural cells from mouse brain tissue in combination with the use of Anti-AN2 MicroBeads.
- MACS SmartStrainers (70 µm) (# 130-098-462) to remove cell clumps.
- 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) 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

Isolation of mouse AN2$^+$ cells from dissociated mouse brain tissue

For the preparation of single-cell suspensions from neural tissues refer to data sheet of 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.

2.2 Antigen re-expression

The AN2 antigen shows papain as well as trypsin sensitivity. Therefore, the single-cell suspension has to be incubated under slow continuous rotation using the MACSmix Tube Rotator for 3 hours at 37 °C in the Re-expression and Cultivation Medium (refer to chapter 1.4) to re-express the AN2 antigen after enzymatic treatment. Use 5 mL of re-expression medium per 1×10^7 cells.

Cells should be processed immediately after re-expression for magnetic labeling and separation.

▲ **Note:** After re-expression APC autofluorescence has been observed. Therefore, it is recommended not to use APC-conjugated antibodies for flow cytometric analysis after re-expression.

2.3 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 (MACS SmartStrainers (70 µm) # 130-098-462) 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 80 µL of buffer per 10^7 total cells.
4. Add 10 µL of FcR Blocking Reagent per 10^7 nucleated cells.
5. Mix well. Do not vortex. Incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 10 µL of Anti-AN2 MicroBeads per 10^7 total cells.
7. Mix well. Do not vortex. Incubate for 15 minutes in the refrigerator (2–8 °C).
8. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend up to 10^7 cells in 500 µL of buffer.

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

10. Proceed to magnetic separation (2.4).

2.4 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of AN2$^+$ cells. For details refer to the table in section 1.4.

▲ **Note:** MS Columns are recommended for highest purity of AN2$^+$ cells, LS Columns are recommended for highest recovery of AN2$^+$ cells.

▲ 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

   ▲ **Note:** Elution of the cells from the column after the separation should be performed with Re-expression and Cultivation 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:

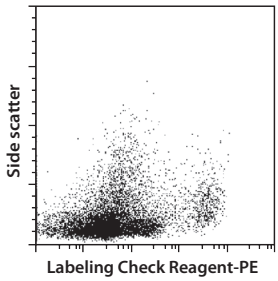
Positive selection: Possel
Collect positive fraction in row C of the tube rack.

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

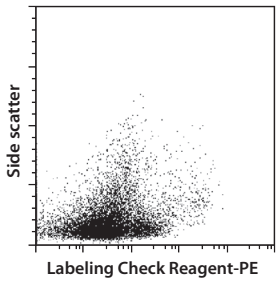
3. Example of a separation using Anti-AN2 MicroBeads

$AN2^+$ cells were isolated from day 6 postnatal CD1 mouse brain. A single-cell suspension was prepared using the Neural Tissue Dissociation Kit (P) and the gentleMACS Dissociator. After re-expression of the AN2 antigen, FcR Blocking Reagent, Anti-AN2 MicroBeads, a MiniMACS™ Separator, and an MS Column were used for the separation of $AN2^+$ cells. Cells were fluorescently stained with Labeling Check Reagent-PE (# 130-095-228) and 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.

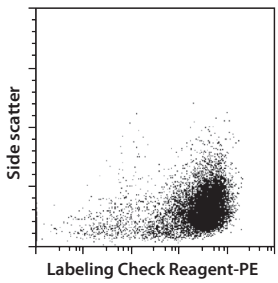
Before separation



$AN2^-$ cells



$AN2^+$ cells



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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