Miltenyi Biotec

Anti-ACSA-2 MicroBead Kit
mouse

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 MicroBeads conjugated to monoclonal anti-ACSA-2 antibodies (isotype: rat IgG2b) and FcR Blocking Reagent, mouse.

Product	Order no.
50 µL Anti-ACSA-2 MicroBeads, mouse and 50 µL FcR Blocking Reagent, mouse	130-097-679
1 mL Anti-ACSA-2 MicroBeads, mouse and 1 mL FcR Blocking Reagent, mouse	130-097-678

Capacity For 5×10^7 total cells, up to 5 separations (# 130-097-679)
or
for 10^9 total cells, up to 100 separations (# 130-097-678).

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, Fc receptors are blocked with FcR Blocking Reagent, mouse. Then, the ACSA-2^+ cells are magnetically labeled with Anti-ACSA-2 MicroBeads. The cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled ACSA-2^+ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of ACSA-2^+ cells. After removing the column from the magnetic field, the magnetically retained ACSA-2^+ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected fraction containing the ACSA-2^+ cells can be separated over a second column.

1.2 Background information

Anti-ACSA-2 MicroBeads (ACSA-2: **a**strocyte **c**ell **s**urface **a**ntigen-2) have been developed for the isolation of astrocytes from cell suspensions of mouse neural tissue based on the expression of the ACSA-2 antigen.
The ACSA-2 antigen is expressed specifically on astrocytes in a pattern similar to GLAST. Therefore, ACSA-2 is a specific marker for astrocytes in the central nervous system (CNS). The percentage of ACSA-2^+ astrocytes differs according to the mouse age and the brain region used for cell isolation.
Anti-ACSA-2 MicroBeads allow for the isolation of astrocytes from all mouse age stages.

1.3 Applications

- Positive selection or depletion of cells expressing the mouse ACSA-2 antigen.
- Isolation or depletion of ASCA-2^+ astrocytes from dissociated mouse brain tissue.

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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS).

  ▲ **Note:** Always use freshly prepared buffer. Do **not use** autoMACS Running Buffer or MACSQuant Running Buffer as they contain a small amount of sodium azide that could affect the results.

  ▲ **Note:** (Optional) Use AstroMACS Separation Buffer (# 130-117-336) instead. Keep buffer cold (2–8 °C).

- 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 from mice up to P7.

  ▲ **Note:** If additional cell surface epitopes of interest are papain-sensitive, the Neural Tissue Dissociation Kit (T) (# 130-093-231) can be used.

- Adult Brain Dissociation Kit, mouse and rat (# 130-107-677) is recommended for the generation of single-cell suspensions of

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neural cells from mouse brain tissue derived from mice older than P7.

- Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps.
- MACS Columns and MACS Separators: ACSA-2$^+$ 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.

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

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

- (Optional) Fluorochrome-conjugated ACSA-2 antibodies for flow cytometric analysis, e.g., ACSA-2 Antibody, anti-mouse, PE. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) MACS® Neuro Medium (# 130-093-570) and MACS NeuroBrew®-21 (# 130-093-566) for cultivation.
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), gentleMACS Octo Dissociator with Heaters (# 130-096-427), and gentleMACS C Tubes (# 130-093-237, # 130-096-334).

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) or Adult Brain Dissociation Kit, which can be used in combination with the gentleMACS Dissociator.

▲ **Note:** If additional cell surface epitopes of interest are papain-sensitive, the Neural Tissue Dissociation Kit (T) can be used.

A special protocol for the isolation of astrocytes "Isolation of astrocytes from adult mouse brain" is available at www.miltenyibiotec.com/130-097-678 in the Resources tab.

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)) 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. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. Add 10 µL of FcR Blocking Reagent per 10^7 total cells.
5. Mix well. Do not vortex. Incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 10 µL of Anti-ACSA-2 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 (2.3 or 2.4).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of ACSA-2$^+$ 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. This is the ACSA-2-negative cell fraction.

   MS: 3×500 µL LS: 3×3 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. This is the ACSA-2-positive cell fraction.

   MS: 1 mL LS: 5 mL

7. (Optional) To increase the purity of ACSA-2$^+$ cells, it is recommended to enrich the positive fraction 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 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.

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.

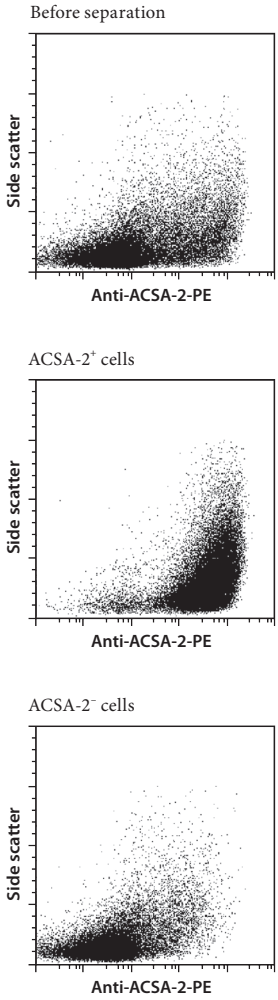
3. For a standard separation choose one of the following programs:

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

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

3. Example of a separation using the Anti-ACSA-2 MicroBead Kit

ACSA-2$^+$ cells were isolated from P4 CD-1 mouse brain tissue using the Neural Tissue Dissociation Kit (P), the gentleMACS Dissociator, FcR Blocking Reagent, mouse, Anti-ACSA-2 MicroBeads, a MiniMACS™ Separator, and an MS Column. Cells were fluorecently stained with ACSA-2 Antibody, anti-mouse, PE 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.



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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