Miltenyi Biotec

Anti-GLAST (ACSA-1) MicroBead Kit
human, mouse, rat

2× 1 mL	130-095-826
2× 100 µL	130-095-825

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-GLAST (ACSA-1)-Biotin, human, mouse, rat:** monoclonal Anti-GLAST (ACSA-1) antibody conjugated to biotin (isotype: mouse IgG2a). **1 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1). or **100 µL Anti-GLAST (ACSA-1)-Biotin, human, mouse, rat – small size:** monoclonal Anti-GLAST (ACSA-1) antibody conjugated to biotin (isotype: mouse IgG2a). **100 µL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1).
Capacity	2× 1 mL for $5×10^8$ total cells, up to 50 separations. or 2× 100 µL for $5×10^7$ total cells, up to 5 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.

Cross-reactivity: The Anti-GLAST (ACSA-1) MicroBead Kit has been tested to react with mouse and rat cells. Cross-reactiviy of the Anti-GLAST (ACSA-1) antibody for human cells has been successfully tested by immunohistochemistry.

1.1 Principle of the MACS® Separation

First, the GLAST$^+$ cells are indirectly magnetically labeled with Anti-GLAST (ACSA-1)-Biotin antibodies and Anti-Biotin 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 GLAST$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of GLAST$^+$ cells. After removing the column from the magnetic field, the magnetically retained GLAST$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the GLAST$^+$ cells can be separated over a second column.

1.2 Background information

The Anti-GLAST (ACSA-1) MicroBead Kit (ASCA-1: **a**strocyte **c**ell **s**urface **a**ntigen-1) has been developed for the separation of astrocytes based on the expression of GLAST.

The Anti-GLAST (ACSA-1) antibody is specific for an extracellular epitope of the astrocyte specific transmembrane glycoproteine GLAST. GLAST is a Na$^+$-dependent L-glutamate transporter, which is important for removing the excitatory neurotransmitter L-glutamate from the extracellular space to maintain normal physiological levels.[1,2]

Besides GLT-1, GLAST is the most abundant glutamate transporter and is predominantly expressed by astrocytes in the developing and neonatal mammalian central nervous system. In addition, radial glia, which belong to the astrocyte lineage and play important roles in development, are known to express GLAST. Postnatally radial glia only persist in a few regions, such as Bergmann glia in the cerebellum, Müller glia in the retina, and radial glia in the dentate gyrus of the adult hippocampus.[3,4]

The isolation of GLAST$^+$ cells was tested particularly on P5–7 (postnatal day 5–7) dissociated mouse brain tissue derived from CD1 mice, containing approximately 12–18% GLAST$^+$ cells.

1.3 Applications

- Positive selection of cells expressing GLAST.
- Isolation of GLAST$^+$ astrocytes from dissociated mouse or rat brain tissue (derived from rodents younger than eight days (<P8)).

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).

- MACS Columns and MACS Separators: GLAST $^+$ cells can be enriched by using MS or LS Columns. Positive selection 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
autoMACS	5×10^7	1×10^8	autoMACS Pro
Multi-24	2×10^7	4×10^7	MultiMACS Cell24

- Neural Tissue Dissociation Kit (T) (# 130-093-231) for the generation of single-cell suspensions of neural cells from mouse brain tissue.

  ▲ **Note:** The GLAST-epitope shows papain sensitivity, therefore it is recommended to use a trypsin-based dissociation.

  ▲ **Note:** Use a 70 µm cell strainer for filtration of dissociated brain tissue.

- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427).

- (Optional) FcR Blocking Reagent, mouse (#130-092-575) to avoid Fc receptor–mediated antibody labeling.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Anti-GLAST (ACSA-1)-PE (# 130-095-821) or Anti-GLAST (ACSA-1)-APC (# 130-095-814). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

- (Optional) Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.

- (Optional) MACS Neuro Medium (# 130-093-570) and MACS NeuroBrew-21 (# 130-093-566) for the cultivation of astrocytes.

2. Protocol

2.1 Sample preparation

When working with neural tissues, prepare a single-cell suspension using manual methods or the gentleMACS Dissociators. As the GLAST-epitope shows papain sensitivity, it is recommended to use a trypsin-based dissociation.

For details refer to the protocols section at 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. For best performance it is recommended to use at least 5×10^6 cells. When working with higher cell numbers than 10^7, 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 10 minutes. Aspirate supernatant completely.

3. Add 80 µL of buffer per 10^7 total cells to the cell pellet.

   ▲ **Note:** If FcR Blocking Reagent, mouse is being used, add 70 µL of buffer and 10 µL FcR Blocking Reagent, mouse per 10^7 nucleated cells. Mix well. Do not vortex. Incubate for 10 minutes in the refrigerator (2–8 °C).

4. Add 20 µL of Anti-GLAST (ACSA-1)-Biotin per 10^7 total cells.

5. Mix well. Do not vortex. Incubate for 10 minutes in the refrigerator (2–8 °C).

6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

7. Add 80 µL of buffer per 10^7 total cells to the cell pellet.

8. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.

9. Mix well. Do not vortex. Incubate for 15 minutes in the refrigerator (2–8 °C).

10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

11. Resuspend up to 10^7 cells in 500 µL of buffer.

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

12. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Always wait until the column reservoir is empty before proceeding to the next step.

▲ To achieve highest purities, purification of GLAST $^+$ cells should be performed with two consecutive MS Column runs.

▲ Use of LS Columns may result in a 5–10% lower purity in comparison to the use of MS Columns.

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

7. (Optional) To increase the purity of GLAST$^+$ cells, the eluted fraction can be enriched over a second, freshly prepared 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 times of cells in PBS/BSA buffer to a minimum.

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 the following program:

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

3. Example of a separation using the Anti-GLAST (ACSA-1) MicroBead Kit

GLAST$^+$ cells (positive fraction) were isolated from day 7 postnatal mouse brain tissue using the Neural Tissue Dissociation Kit (T), the gentleMACS Dissociator, FcR Blocking Reagent, mouse, the Anti-GLAST (ACSA-1) MicroBead Kit, a MiniMACS™ Separator, and an MS Column. Cells were fluorescently stained with Anti-GLAST (ACSA-1)-APC 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

Positive fraction

Negative fraction
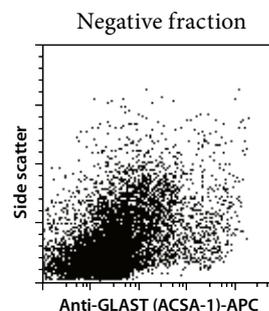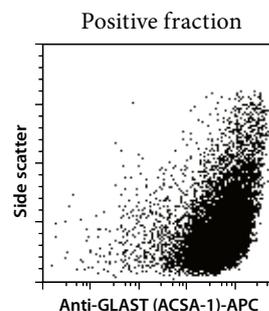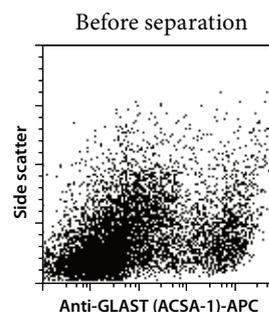
4. References

1. Storck, T. *et al.* (1992) Structure, expression, and functional analysis of a Na(+)-dependent glutamate/aspartate transporter from rat brain. Proc. Natl. Acad. Sci. USA 89: 10955–10959.

2. Wahle, S. and Stoffel, W. (1996) Membrane topology of the high-affinity L-glutamate transporter (GLAST-1) of the central nervous system. J. Cell Biol. 135: 1867–1877.

3. Kimelberg, H.K. (2004) The problem of astrocyte identity. Neurochem. Int. 45: 191–202.

4. Kriegstein, A.R. and Götz, M. (2003) Radial glia diversity: a matter of cell fate. Glia 43: 37–43.

All protocols and data sheets are available at **www.miltenyibiotec.com**.

Warranty