Anti-GLAST (ACSA-1) antibodies
human, mouse, rat

Contents

1. Description

Components 1 mL monoclonal Anti-GLAST (ACSA-1) antibodies, human, mouse, rat conjugated to various dyes.

PE	130-095-821
APC	130-095-814
Biotin	130-095-815
pure	130-095-822

Clone ACSA-1 (isotype: mouse IgG2a).

Capacity 100 tests or up to 10^8 total cells.

The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.

Product format Antibodies 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) antibody has been tested to react with mouse and rat cells by flow cytometric analysis and with mouse and human cells by immunohistochemistry.

1.1 Background information

The Anti-GLAST (ACSA-1) antibodies (ASCA-1: **a**strocyte **c**ell **s**urface **a**ntigen-1) have been developed for the detection of astrocytes based on the expression of GLAST.
The antibody is specific for an extracellular epitope of the astrocyte specific transmembrane glycoproteine GLAST in the central nervous system (CNS). GLAST (EAAT1) 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 CNS. 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]

1.2 Applications

- Identification and enumeration of $GLAST^+$ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. $GLAST^+$ cells can be isolated by using the Anti-GLAST (ACSA-1) MicroBead Kit, human, mouse, rat (# 130-095-826).

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-GLAST (ACSA-1) conjugates is **1:11 for up to 10^6 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For Anti-GLAST (ACSA-1) MicroBead Kit–labeled cells use the same dilution.

The pure antibody is tested to work on cryosections. The optimal antibody concentration is approximately 2 µg/mL.

1.4 Reagent 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).

  ▲ **Note:** BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum (FBS).
- (Optional) 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)
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., APC (# 130-090-856) as secondary antibody reagent in combination with Anti-GLAST (ACSA-1)-Biotin.
- (Optional) Mouse IgG2a isotype control antibodies conjugated to, e.g., APC (# 130-091-836). For more information about isotype control antibodies refer to www. miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.

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2. General protocol for immunofluorescent staining

▲ Volumes given below are for **up to 10^6** nucleated cells. When working with fewer than 10^6 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 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

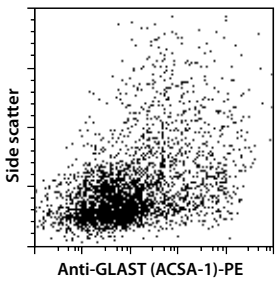
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Add 100 µL of buffer per 10^6 nucleated cells to the cell pellet.
   ▲ **Note:** If FcR Blocking Reagent, mouse is being used, add 90 µL of buffer and 10 µL FcR Blocking Reagent, mouse directly before addition of the Anti-GLAST (ACSA-1) antibody per 10^6 nucleated cells.
4. Add 10 µL of the Anti-GLAST (ACSA-1) antibody.
5. Mix well. Do not vortex. Incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-GLAST (ACSA-1)-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with Anti-GLAST (ACSA-1) antibodies

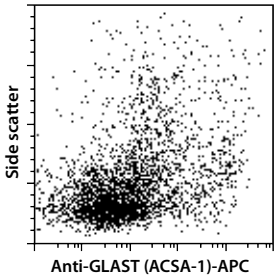
Mouse brain tissue postnatal day 7 was dissociated using the Neural Tissue Dissociation Kit (T) and the gentleMACS Dissociator. Brain cells were stained using Anti-GLAST (ACSA-1) antibodies conjugated to PE (A) or APC (B) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-GLAST (ACSA-1)-Biotin (C) were stained with Anti-Biotin-APC.

Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

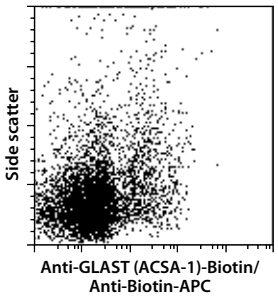
A)



B)



C)



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**.

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.

Warranty

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