Anti-ACSA-2 antibodies, mouse

For research use only

30 µg equal 100 tests, 150 µg equal 500 tests. One test corresponds to labeling of 10 cells.

Product	Content	Order no.
Anti-ACSA-2-Biotin	30 µg in 200 µL	130-116-242
Anti-ACSA-2-FITC	30 µg in 200 µL	130-116-243
Anti-ACSA-2-PE	30 µg in 200 µL	130-116-244
Anti-ACSA-2-PE	150 µg in 1 mL	130-116-141
Anti-ACSA-2-APC	30 µg in 200 µL	130-116-245
Anti-ACSA-2-APC	150 µg in 1 mL	130-116-142
Anti-ACSA-2-PE-Vio615	30 µg in 200 µL	130-116-249
Anti-ACSA-2-PE-Vio615	150 µg in 1 mL	130-116-146
Anti-ACSA-2-PE-Vio770	30 µg in 200 µL	130-116-246
Anti-ACSA-2-APC-Vio770	30 µg in 200 µL	130-116-247
Anti-ACSA-2-VioBright 515	30 µg in 200 µL	130-116-248

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.

Technical data and background information

Antigen	ACSA-2
Clone	REA969
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	ACSB, Acetate CoA ligase, Acyl-activating enzyme 2
Distribution of antigen	astrocytes, CNS cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA969 recognizes the mouse ACSA-2 antigen (ACSA-2: astrocyte cell surface antigen-2), which has been developed for the detection of astrocytes from cell suspensions of mouse neural tissue based on the expression of the ACSA-2 protein. The ACSA-2 antigen is specifically expressed on GLAST (ACSA-1) positive astrocytes and is therefore a specific marker of astrocytes in the developing and neonatal mouse central nervous system (CNS). The percentage of ACSA-2 positive astrocytes differs according to the mouse age and the brain region. Additional information: Clone REA969 displays negligible binding to Fc receptors.

Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2−8 °C).
  Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

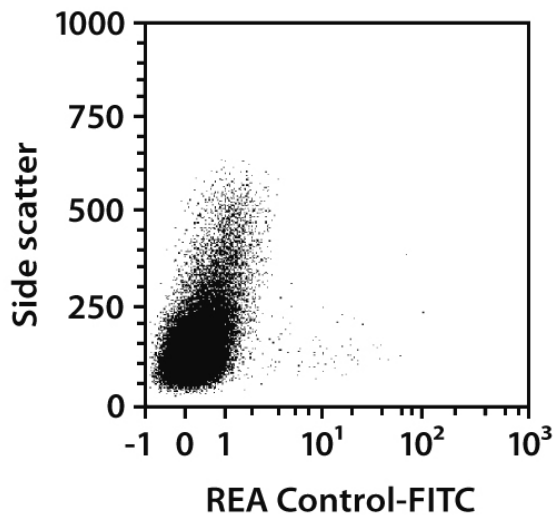
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- 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.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and 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 biotinylated antibody was used, resuspend the cell pellet in buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

Examples of immunofluorescent staining

Brain tissue postnatal day 5 from CD-1®mice was dissociated using the Neural Tissue Dissociation Kit (P) and the gentleMACS™ Dissociator. Brain cells were stained with Anti-ACSA-2 antibodies or with the corresponding REA Control antibodies (left images) 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 or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates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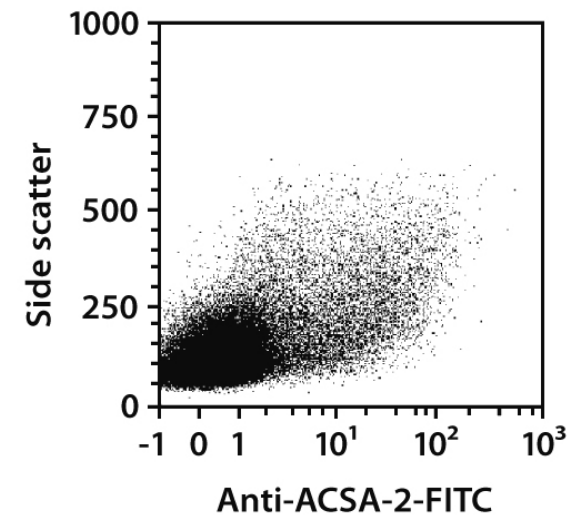
REA Control-FITC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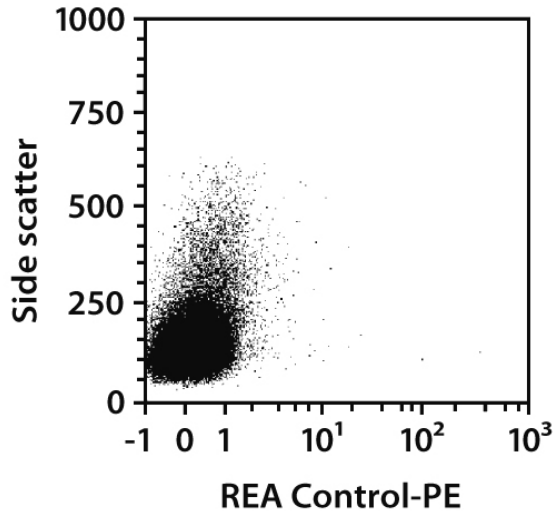
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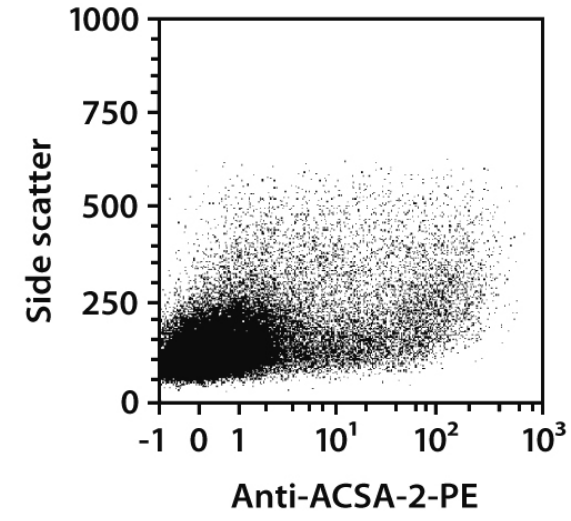
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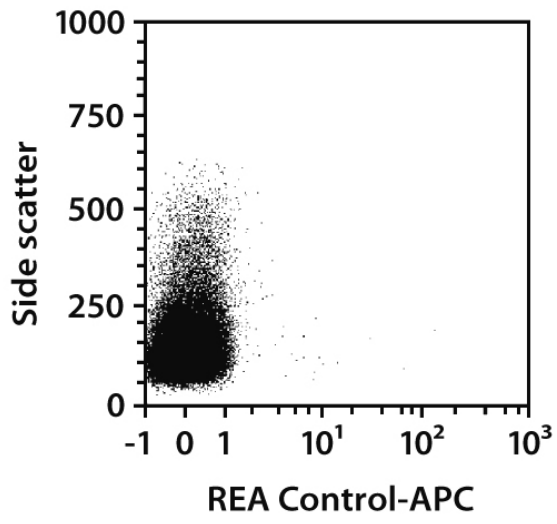
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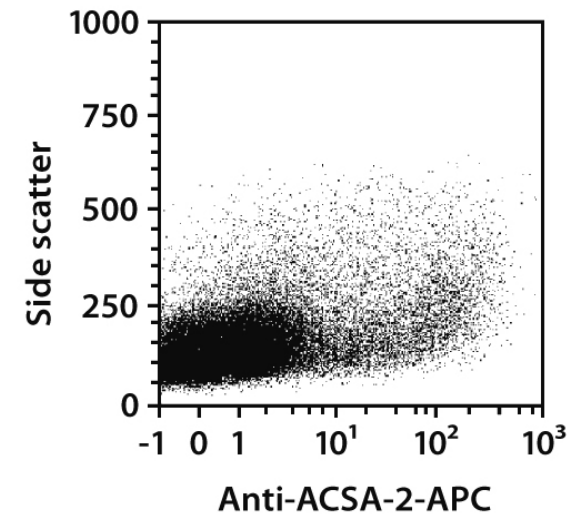
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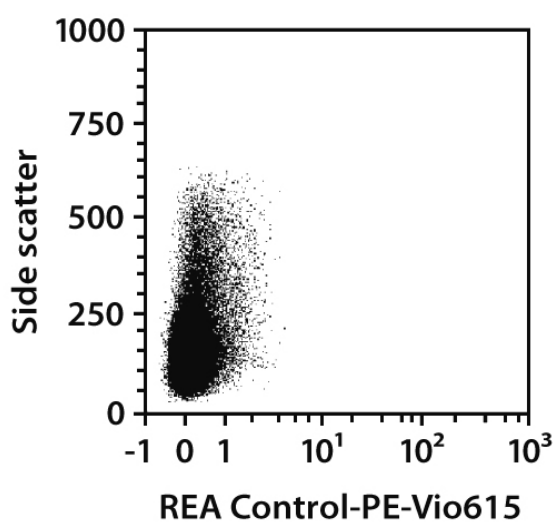
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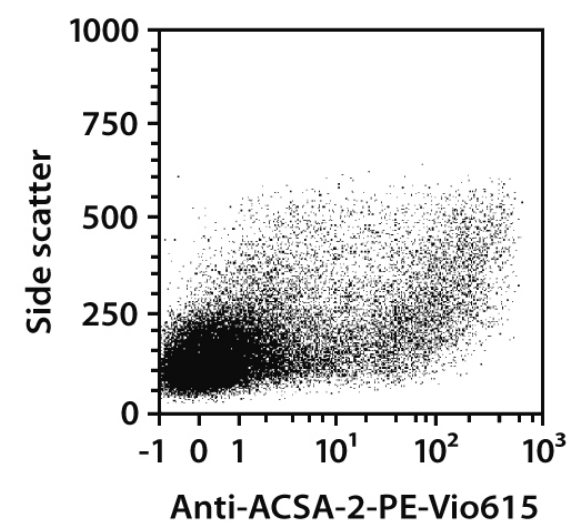
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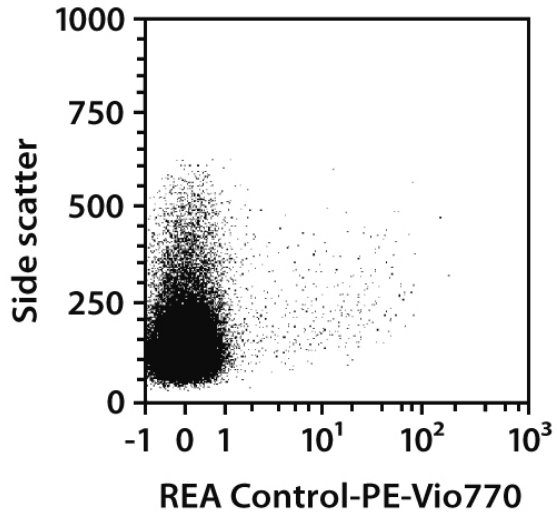
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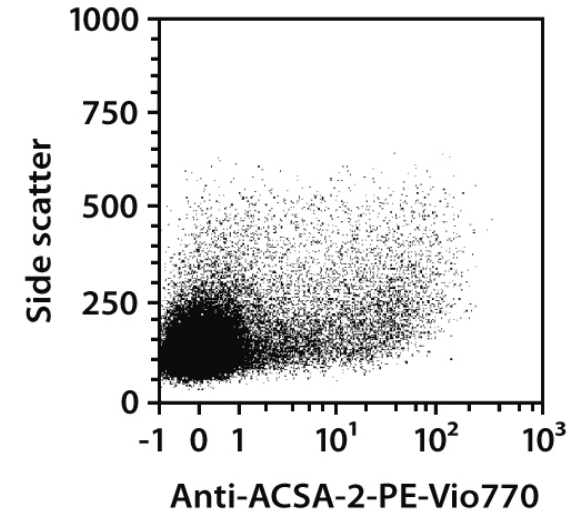
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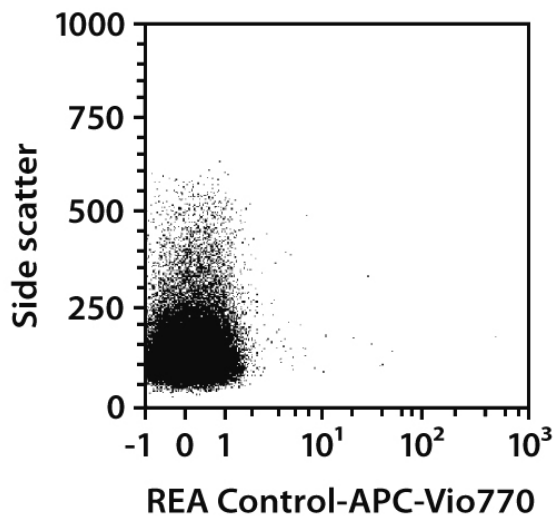

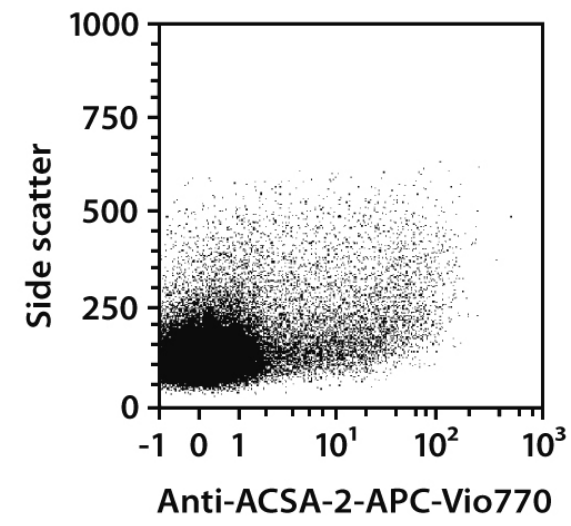

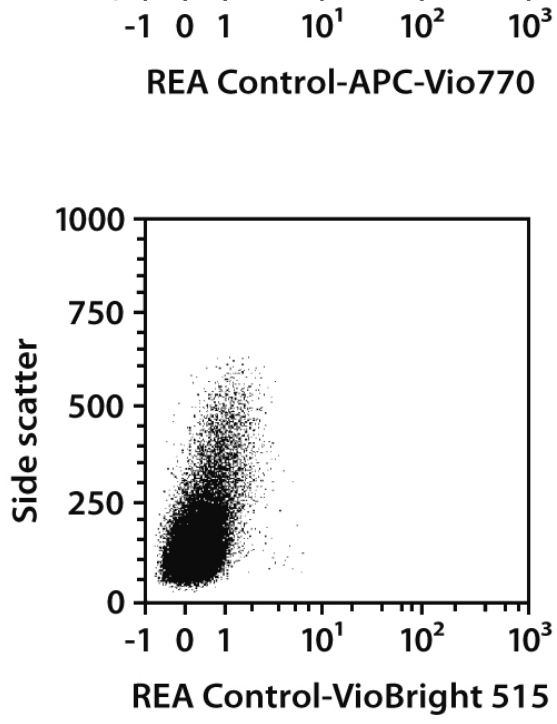

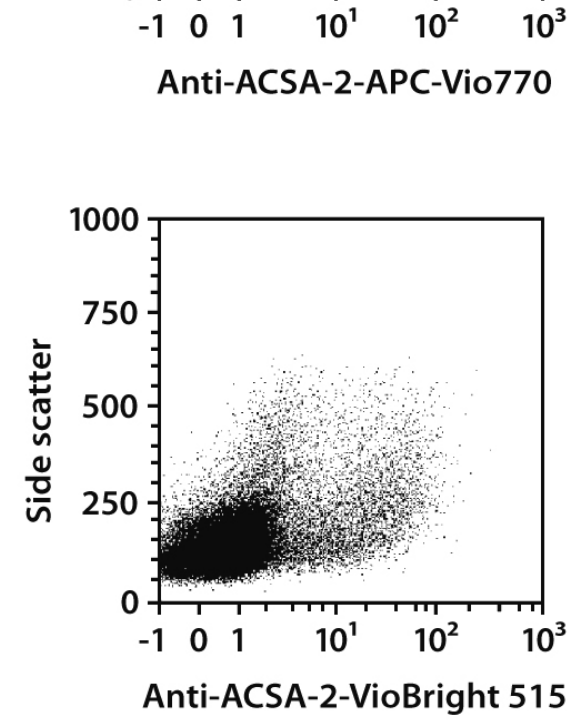

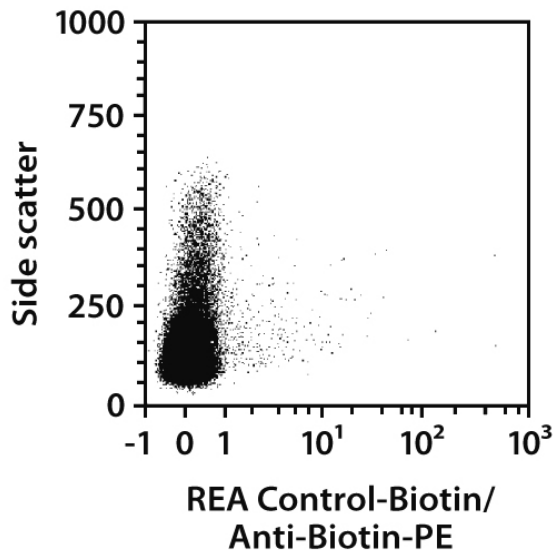

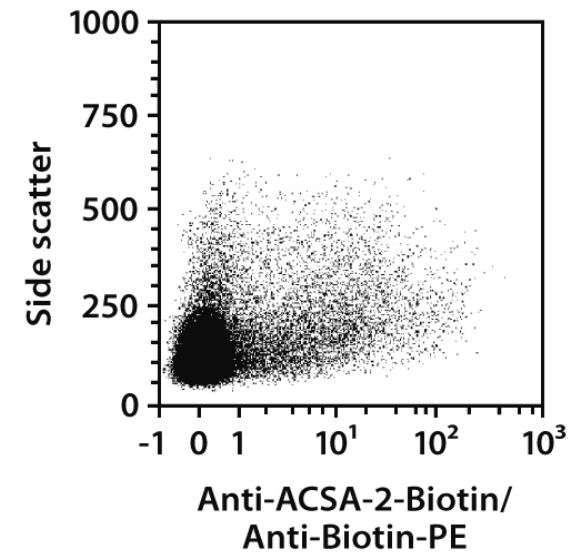

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