

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human AMICA: Supplied as 10 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 401901

Isotype: mouse IgG_{2A}

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

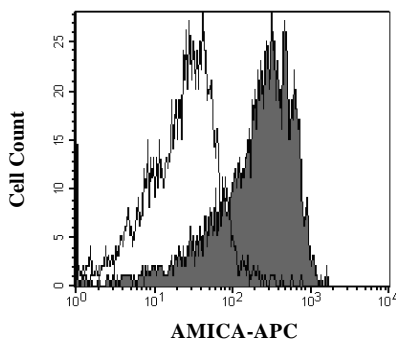
Designed to quantitatively determine the percentage of cells bearing AMICA within a population and qualitatively determine the density of AMICA on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the allophycocyanin-labeled monoclonal antibody, which binds to cells expressing AMICA. Unbound allophycocyanin-conjugated antibody is then washed from the cells. Cells expressing AMICA are fluorescently stained, with the intensity of staining directly proportional to the density of expression of AMICA. Cell surface expression of AMICA is determined by flow cytometry using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

Reagent Preparation

Allophycocyanin-conjugated mouse anti-human AMICA: Use as is; no preparation necessary.



Human monocytes were stained with APC-conjugated anti-human AMICA (Catalog # FAB34491A, filled histogram) or isotype control (Catalog # IC003A, open histogram).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) followed by centrifugation at 500 x g for 5 minutes. 50 µL of packed cells should then be transferred to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA) to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10⁶ cells/mL and 25 µL of cells (1 x 10⁵) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

- 1) Cells should be Fc-blocked by treatment with 1 µg of human IgG/10⁵ cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
- 2) Transfer 25 µL of the Fc-blocked cells (1 x 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of APC-conjugated AMICA reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted AMICA reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
- 6) Finally, resuspend the cells in 200 - 400 µL of PBS buffer for analysis by flow cytometry.
- 7) As a control for this analysis, cells in a separate tube should be treated with APC-labeled mouse IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

Background Information

AMICA (adhesion molecule, interacting with CXADR antigen 1), also known as JAML, is a 65 kDa, heavily glycosylated transmembrane protein that belongs to the junctional adhesion molecule (JAM) subset of the immunoglobulin superfamily (1). JAM family molecules contribute to intercellular connections within epithelial and endothelial cell layers, and mediate their interactions with various hemopoietic cells (1). The human AMICA cDNA encodes a 384 amino acid (aa) precursor that includes a 19 aa signal sequence, a 256 aa extracellular domain (ECD) with two Ig-like domains, a 21 aa transmembrane segment, and a 98 aa cytoplasmic domain (2). Alternative splicing may generate isoforms with N- and C-terminal deletions. In contrast to other JAM family proteins, AMICA does not contain a cytoplasmic PDZ-binding motif (3). Within the ECD, human AMICA shares 58% and 63% aa sequence identity with mouse and rat AMICA, respectively. It shares 18% - 20% aa sequence identity with the ECDs of human JAM-A, -B, -C, and JAM4. AMICA is expressed on the surface of granulocytes and monocytes and is up-regulated during the differentiation of myeloid leukemia cells (2, 3). A motif in the ECD, which promotes dimerization of other JAM family proteins, is required for surface localization of AMICA (2). AMICA mediates the adhesion of monocytes to endothelial cells (2) and neutrophil migration across epithelial cell monolayers (3). This latter function involves specific interactions of AMICA with coxsackie virus and adenovirus receptor (CXADR) in epithelial tight junctions (3). In particular, the membrane proximal Ig-like domain of AMICA binds the membrane-distal Ig-like domain of CXADR (3). AMICA does not appear to interact homophilically, as neutrophils adhere to immobilized CXADR but not to immobilized AMICA (3).

References

1. Mandell, K.J. and Parkos, C.A., 2005, *Adv. Drug Deliv. Rev.* **57**:857.
2. Moog-Lutz, C. *et al.*, 2003, *Blood* **102**:3371.
3. Zen, K. *et al.*, 2005, *Mol. Biol. Cell* **16**:2694.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.