Monoclonal Anti-human ALCAM (CD166)-Phycoerythrin

Catalog Number: FAB6561P
Lot Number: LAR03
100 tests

Reagent Information

Phycoerythrin-conjugated monoclonal anti-human ALCAM (CD166): Supplied as 35 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 105902

Ig class: mouse IgG_1

Additional Reagents Required

- 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 cell surface ALCAM within a population and qualitatively determine the density of this receptor on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody that binds to the cells expressing ALCAM (CD166). Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing ALCAM are fluorescently stained, with the intensity of staining directly proportional to the density of ALCAM. Cell surface expression of ALCAM is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Phycoerythrin-conjugated mouse anti-human ALCAM: Use as is; no preparation is necessary.

Sample Preparation

Peripheral blood cells: Whole blood should be collected in 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) by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Blood cells 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), as described above, 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) are transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate 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 to be used for staining with the antibody may be first Fc-blocked by treatment with 1 μg of mouse or human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of phycoerythrin-conjugated anti-human ALCAM reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-ALCAM reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Human Erythrocyte Lysing Kit, Catalog # WL1000*).
6) Resuspend the cells in 200 - 400 μL of PBS buffer for final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with phycoerythrin-labeled mouse IgG_1 antibody.

This procedure may need modification, depending upon final utilization.

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ALCAM (CD166)

Activated Leukocyte Cell Adhesion Molecule (ALCAM), also known as CD166, SB-10, and MEMD, belongs to a subgroup of the immunoglobulin supergene family that exhibits five extra-cellular immunoglobulin-like domains (VVC2C2C2). ALCAM is a type I transmembrane glycoprotein (1) capable of mediating both homophilic (ALCAM-ALCAM) and heterophilic (ALCAM-CD6) cell-cell interactions (1 - 5). Human and mouse ALCAM show 93% amino acid homology and mediate cross-species cell-cell adhesion (6).

ALCAM is reportedly expressed on thymic epithelial cells (1, 2), activated T and B cells (1, 2), monocytes (1, 2), endothelial cells (2), neurons (2), mesenchymal stem cells (7), bone marrow stromal cells (2), osteoblastic cell lines (8), and on highly metastatic melanoma cells (9), plus other tumor cell types (1, 2). ALCAM expression requires alpha-catenin for localization from the cytoplasm to the cell surface (10). Some cytokines have been shown to augment ALCAM expression. For example, IL-3, M-CSF, and GM-CSF all increase the level of ALCAM on monocytes (11).

It has been suggested that ALCAM-CD6 interactions are important for T cell development and regulation of T cell function (1, 2). In addition to CD6, ALCAM has been demonstrated to bind neural-glial cell adhesion molecule (NgCAM) (12) and other neural proteins (2, 12), suggesting an as yet unidentified role for ALCAM in the nervous system. ALCAM also has been described as having a role in such diverse functions as hematopoiesis, vasculoangiogenesis (13), bone and bone marrow development (14), and tumor cell migration (9, 15). Of clinical interest are observations that ALCAM plays a role in rheumatoid arthritis (11) and as a potential marker of tumor progression in malignant melanoma (16).

References

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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.