Monoclonal Anti-human JAM-C-Phycoerythrin

Catalog Number: FAB11891P
Lot Number: LOD02
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human JAM-C: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 208212

Isotype: mouse IgG_{2B}

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

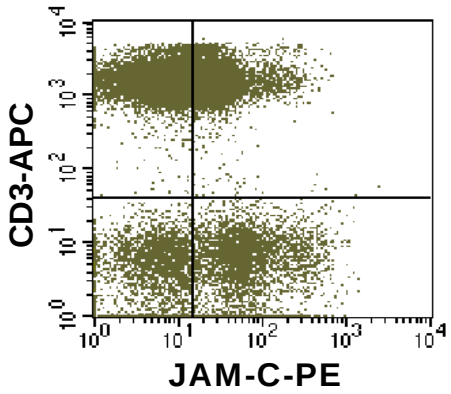
Designed to quantitatively determine the percentage of cells bearing JAM-C within a population and qualitatively determine the density of JAM-C on cell surfaces by flow cytometry.

Principle of the Test

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

Reagent Preparation

PE-conjugated mouse anti-human JAM-C: Use as is; no preparation necessary.



Whole blood lymphocytes double stained with PE-conjugated anti-human JAM-C (Catalog # FAB11891P) and APC-conjugated anti-human CD3 (Catalog # FAB100A).

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) by centrifugation at 500 x g for 5 minutes. Transfer 50 μL of packed cells 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), 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 x 10^6 cells/mL and 25 μL of cells (1 x 10^5) 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 should be Fc-blocked by treatment with 1 μg of human IgG/10^5 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 10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μl of PE-conjugated anti-JAM-C reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-JAM-C 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 final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with PE-labeled mouse IgG_{2B} antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

The family of **J**unctional **A**dhesion **M**olecules (JAM), comprising at least three members, are type I transmembrane receptors belonging to the immunoglobulin superfamily (1, 2). These proteins are localized in the tight junctions between endothelial cells or epithelial cells. Some family members are also found on blood leukocytes and platelets.

Human JAM-C is a 43 kDa molecule that shares 86% amino acid sequence identity with its mouse homologue. It also shares approximately 36% and 32% amino acid sequence homology with human JAM-B and JAM-A, respectively (3 - 5). Human JAM-C shows widespread tissue expression and the highest levels are found in the placenta, brain, kidney, and heart (3). JAM-C is also expressed on platelets, T cells and NK cells (3 - 5). JAM-C binds to JAM-B to facilitate the interactions between JAM-B and the integrin $\alpha_4\beta_1$ (6). This heterotypic interaction between leukocyte JAM-C and endothelial JAM-B may play a role in regulating leukocyte transmigration (5). On platelets, JAM-C is a counter-receptor for the leukocyte integrin Mac-1 (CD11b/CD18) (7). JAM-C has also been identified as a strong candidate gene for hypoplastic left heart syndrome (8).

Note: *The nomenclature used for the JAM family proteins is confusing. VE-JAM has been referred to in the literature variously as JAM-B or JAM-C. Until further clarification, R&D Systems has adopted the nomenclature where both mouse and human VE-JAM are referred to as JAM-B. Under this system, JAM-C refers to the protein encoded by the gene localized to human chromosome 11.*

References

1. Chavakis, T. *et al*. (2003) Thromb. Haemost. **89**:13.
2. Aurrand-Lions, M. *et al*. (2001) Blood **98**:3699.
3. Arrate, M.P. *et al*. (2001) J. Biol. Chem. **276**:45826.
4. Liang, T. *et al.* (2002) J. Immunol. **168**:1618.
5. Johnson-Leger, C. *et al*. (2002) Blood **100**:25793.
6. Cunningham, A. *et al.* (2002) J. Biol. Chem. **277**:27589.
7. Santoso, S. *et al*. (2002) J. Exp. Med. **196**:679.
8. Phillips, H.M. *et al*. (2002) Genomics **79**:475.

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.