Monoclonal Anti-human CD9-Fluorescein

Catalog Number: FAB1880F
Lot Number: LKM04

Reagents Provided

This kit provides enough reagents for a total of 100 reactions.

Clone #: 209306

Isotype: mouse IgG_{2B}

Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human CD9: Supplied as 50 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

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

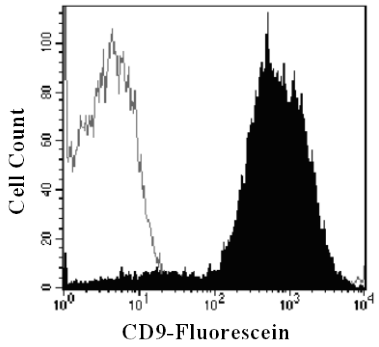
Designed to quantitatively determine the percentage of cells bearing CD9 within a population and qualitatively determine the density of CD9 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody, which binds to cells expressing CD9. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing the CD9 structure are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD9. Cell surface expression of CD9 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated mouse anti-human CD9: Use as is; no preparation necessary.



Human peripheral blood platelets stained with CFS-conjugated anti-human CD9 (Catalog # FAB1880F, filled histogram) or CFS-conjugated isotype control (Catalog # IC0041F, 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) 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) 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 (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of fluorescein-conjugated anti-CD9 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-CD9 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 fluorescein-labeled mouse IgG_{2B} antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

CD9 is a 24 kDa cell surface molecule that is a member of the tetraspan superfamily of proteins (1, 2). Also known as MRP-1 (motility-related protein 1), or as the platelet and leukemia-associated cell surface antigen p24 (3-5), CD9 was originally described on leukemic cells and developing B cells (6). Cloned from a megakaryocyte-derived cDNA library (6), human CD9 is expressed on platelets, early B cells, eosinophils, basophils and activated T cells (6). CD9 is expressed on the surface of acute lymphoblastic leukemia cells and is shed into the plasma of patients (7).

Members of the tetraspan family, including CD9, homodimerize and form complexes with each other (8, 9). In addition, CD9 often is associated with class II MHC and integrin molecules (9). Specifically, CD9 interactions with $\alpha 4\beta 1$ and $\alpha 5\beta 1$ have been observed in several cell lines (10). Functionally, the interaction of CD9 with integrin molecules lends support to hypotheses that CD9 plays a role in intracellular cytoskeleton signalling, cell adhesion and cell motility (9, 10). Finally, CD9 has been shown to have a co-stimulatory function for T cells (11, 12) adding evidence that CD9 may have a role in cell signaling.

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.