Monoclonal Anti-mouse CD48/SLAMF2-Phycoerythrin

Catalog Number: FAB3327P
Lot Number: AAJL02
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-mouse CD48/SLAMF2: Supplied as 50 μg of antibody in 1 mL PBS containing 0.09% sodium azide.

Clone #: 331504

Isotype: rat IgG_1

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

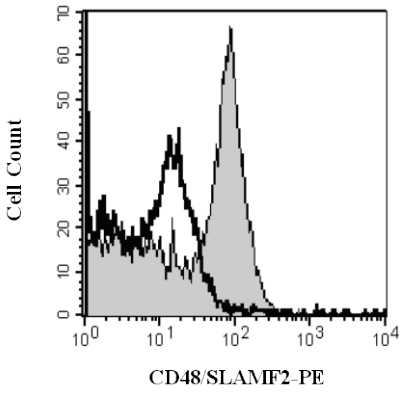
Designed to quantitatively determine the percentage of cells bearing CD48/SLAMF2 within a population and qualitatively determine the density of CD48/SLAMF2 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing CD48/SLAMF2. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing CD48/SLAMF2 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD48/SLAMF2. Cell surface expression of CD48/SLAMF2 is determined by flow cytometric analysis using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.

Reagent Preparation

Phycoerythrin-conjugated rat anti-mouse CD48/SLAMF2: Use as is; no preparation necessary.



Mouse splenocytes were stained with anti-mouse CD48/SLAMF2 (Catalog # FAB3327P, filled histogram) or isotype control (Catalog # IC005P, 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 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 mouse 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 (up to 1 x 10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated CD48/SLAMF2 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted CD48/SLAMF2 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 rat IgG_1 antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

CD48, also known as BLAST-1, BCM-1, and SLAMF2, is a 65 kDa GPI-linked protein in the CD2 family of immunoglobulin superfamily proteins.[1 - 3] The mouse CD48 cDNA encodes a 240 amino acid (aa) precursor that includes a 22 aa signal sequence, a 195 aa mature protein that contains one Ig-like V-type domain and one Ig-like C2-type domain, and a 23 aa C-terminal propeptide.[4] A soluble form of CD48 has been detected in the serum of lymphoid leukemia and arthritis patients.[5] Mouse CD48 shares 51% and 68% aa sequence identity with human and rat CD48, respectively. It shares 18% - 26% aa sequence identity with comparable regions of mouse 2B4, BLAME, CD2F-10, CD84, CD229, CRACC, NTB-A, and SLAM. CD48 is expressed on most lineage-committed hematopoietic cells but not on hematopoietic stem cells or multipotent hematopoietic progenitors.[4,6] Among dendritic cells (DC), CD48 is selectively expressed on circulating myeloid DC and resident bone marrow and thymus DC.[7] CD2, 2B4, and heparan sulfate function as CD48 ligands.[8 - 10] CD48 is competent to transduce signals and can also trigger signaling through CD2 or 2B4.[8, 11] CD48-CD2 interactions promote T cell activation and class switching to IgG_{2a} in B cells.[8, 12] High affinity CD48-2B4 interactions can either promote or inhibit NK cell and cytotoxic T cell (CTL) activation.[7, 11, 13, 14] CD48-2B4 ligation does not directly trigger CTL activity but enhances signaling from the T cell receptor.[13] CD48-2B4 mediated inhibition of NK cell activity is distinct from MHC I-restricted mechanisms.[15] CD48 expressed on NK cells is coactivating, whereas CD48 expressed on other cell types inhibits NK cell activation.[14] Both CD48 expressing and nonexpressing cells can be targets of NK cell or CTL-mediated lysis.[13, 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.