Monoclonal Anti-human CD47-Allophycocyanin

Catalog Number: FAB4670A
Lot Number: AARQ01
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human CD47: Supplied as 10 μg of antibody in 1 mL PBS containing 0.09% sodium azide.

Clone #: 472603

Isotype: mouse 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

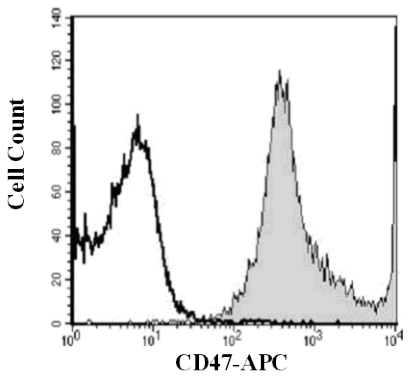
Designed to quantitatively determine the percentage of cells bearing CD47 within a population and qualitatively determine the density of CD47 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the allophycocyanin-labeled monoclonal antibody, which binds to cells expressing CD47. Unbound allophycocyanin-conjugated antibody is then washed from the cells. Cells expressing CD47 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD47. Cell surface expression of CD47 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 CD47: Use as is; no preparation necessary.



Human lymphocytes were stained with APC-conjugated anti-human CD47 (Catalog # FAB4670A, filled histogram) or APC-conjugated isotype control (Catalog # IC002A, 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 4×10^6 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 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 allow 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 (up to 1×10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of APC-conjugated CD47 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted CD47 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, cells in a separate tube should be treated with APC-labeled mouse IgG_1 antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

CD47 (also integrin-associated protein/IAP and OA3) is a variably glycosylated, 40 - 60 kDa atypical member of the Ig superfamily. It is expressed on almost all cell types, including erythrocytes. CD47 binds to TSP-1 and SIRPα, and forms a membrane complex with CD36 and $\alpha_v\beta_3$. Mature human CD47 is a 305 amino acid (aa), five-transmembrane glycoprotein. It contains a 123 aa extracellular region (aa 19 - 141) that is characterized by the presence of a V-type Ig-like domain (aa 19 - 127), and a 34 aa C-terminal cytoplasmic tail that interacts with Giα subunits. Three splice variants occur over aa 293 - 323. Over aa 19 - 139, human CD47 shares 61%, 71%, and 66% aa identity with mouse, porcine, and canine CD47, respectively.

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.