Monoclonal
Anti-human Semaphorin 6A-Allophycocyanin

Catalog Number: FAB1146A
Lot Number: AAWK02
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human Semaphorin 6A: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 169203

Isotype: mouse IgG_{2B}

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

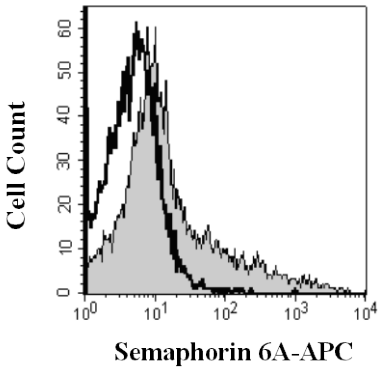
Designed to quantitatively determine the percentage of cells bearing Semaphorin 6A within a population and qualitatively determine the density of Semaphorin 6A on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the allophycocyanin-labeled monoclonal antibody, which binds to cells expressing Semaphorin 6A. Unbound allophycocyanin-conjugated antibody is then washed from the cells. Cells expressing Semaphorin 6A are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Semaphorin 6A. Cell surface expression of Semaphorin 6A 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 Semaphorin 6A: Use as is; no preparation necessary.



Human T cell blasts were stained with APC-conjugated anti-human Semaphorin 6A (Catalog # FAB1146A, filled histogram) or APC-conjugated isotype control (Catalog # IC0041A, 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 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 (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 Semaphorin 6A reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted Semaphorin 6A 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 for this analysis, cells in a separate tube should be treated with APC-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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1-800-343-7475

Background Information

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, NS0-derived, recombinant human Semaphorin 6A (rhSemaphorin 6A; aa 19 - 649; Accession # Q9H2E6) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. Semaphorin 6A is a transmembrane protein expressed in developing neural tissue and involved in axon guidance.

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.