

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human CD69: Supplied as 10 µg of antibody in 1 mL PBS containing up to 0.5% BSA and 0.1% sodium azide

Clone #: 298614

Isotype: mouse IgG_{2A}

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 CD69 within a population and qualitatively determine the density of CD69 on cell surfaces by flow cytometry.

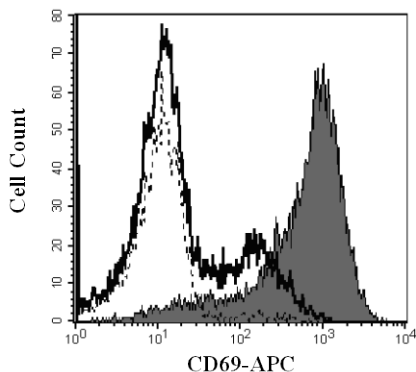
Principle of the Test

Washed cells are incubated with the allophycocyanin-labeled monoclonal antibody, which binds to cells expressing CD69. Unbound allophycocyanin-conjugated antibody is then washed from the cells. Cells expressing CD69 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD69. Cell surface expression of CD69 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 CD69:

Use as is; no preparation necessary.



Human lymphocytes, unactivated (open histogram, bold line) or activated with PHA (filled histogram), were stained with APC-conjugated anti-human CD69 (Catalog # FAB23591A). Activated lymphocytes were also stained with APC-conjugated isotype control antibody (Catalog # IC003A, open histogram dashed line).

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 x 10⁶ cells/mL and 25 µL of cells (1 x 10⁵) 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/10⁵ 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 10⁶ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of APC-conjugated CD69 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted CD69 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 analysis, cells in a separate tube should be treated with APC-labeled mouse IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

Background Information

CD69, also known as Leu 23, AIM, EA-1, and MLR-3, is a type II transmembrane glycoprotein that is expressed on activated T cells, B cells, NK cells, neutrophils, eosinophils, Langerhans cells, and platelets.

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.