Monoclonal Anti-mouse H60-Allophycocyanin

Catalog Number: FAB1155A
Lot Number: LTH02
100 Tests

Reagent Information

Allophycocyanin (APC)-conjugated monoclonal anti-mouse H60: Supplied as 10 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 205326

Ig class: rat IgG_{2A}

Additional Reagents Required

- 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

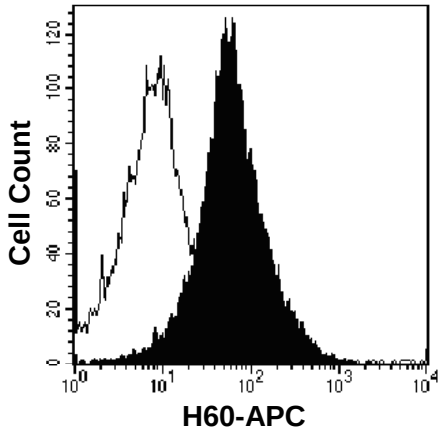
To quantitatively determine the percentage of cells expressing the cell surface protein H60 and qualitatively determine the density of this protein on cell surfaces within a population by flow cytometry.

Principle of the Test

Cells are incubated with the APC-labeled monoclonal antibody, which binds to cells expressing the mouse H60 protein. Unbound APC-conjugated antibody is then washed from the cells. Cells expressing H60 are fluorescently stained, with the intensity of staining directly proportional to the density of H60. Cell surface expression of H60 is determined by flow cytometric analysis 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

APC-conjugated rat anti-mouse H60: Use as is; no preparation necessary.



Mouse monocytic cell line RAW264 stained with APC-conjugated anti-mouse H60 (Catalog # FAB1155A, filled histogram) or isotype control antibody (Catalog # IC006A, open histogram).

Sample Preparation

Tissues: Whole blood should be collected in tubes containing EDTA or heparin as the anticoagulant. Spleen cells should be first mechanically disaggregated into a single cell suspension. 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. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Whole blood cells 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) are 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 to be used for staining with the antibody may be first Fc-blocked by treatment with 1 μg of mouse IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1 x 10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of APC-conjugated anti-mouse H60 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove any unreacted anti-H60 reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Mouse Erythrocyte Lysing Kit, Catalog # WL2000*).
6) 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 APC-labeled rat IgG_{2A} antibody.

This procedure may need to be modified, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

H60 is a type I transmembrane protein distantly related to the mouse MHC class I proteins (1, 2). H60 was originally described as an immunodominant histocompatibility antigen expressed in Balb mice, but not B6 mice (3). H60 functions as a ligand for NKG2D, an activating receptor found on NK cells, some T cell subsets and activated macrophages (1). H60 shares approximately 25% amino acid identity with the Rae-1 family of proteins that also function as ligands for NKGD2 (1). Expression of H60 has been reported in embryonic tissue, spleen, and some transformed cell lines (1, 2). Ectopic expression of H60 on mouse tumor cell lines leads to the *in vivo* rejection of the tumor cells (4). Binding of H60 to NKG2D results in the activation of cytolytic activity and/or cytokine production by the NKG2D-expressing effector cells (1). Thus, H60 ligand-NKG2D receptor interactions are believed to play an important role in innate and adaptive immunity.

References

1. Diefenback, A. *et al.* (2000) Nature Immunol. **1**:119.
2. Cerwenka, A. *et al.* (2000) Immunity **12**:721.
3. Malarkannan, S. *et al.* (1998) J. Immunol. **161**:3501.
4. Diefenback, A. *et al.* (2001) Nature **413**:165.

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.