Monoclonal Anti-human CD90/Thy-1-Allophycocyanin

Catalog Number: FAB2067A
Lot Number: LNW02
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse anti-human CD90/Thy-1: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: Thy-1A1

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

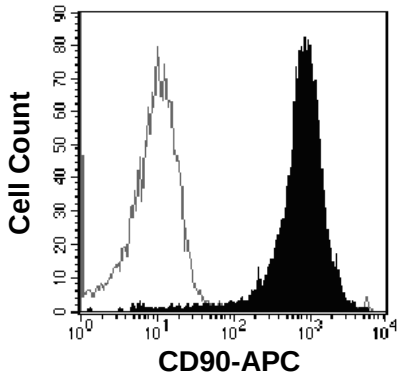
Designed to quantitatively determine the percentage of cells bearing the CD90 antigen (Thy-1) within a population and qualitatively determine the density of CD90 on cell surfaces by flow cytometry.

Principle of the Test

Cells are incubated with the APC-labeled monoclonal antibody, which binds to cells expressing CD90/Thy-1. Unbound APC-conjugated antibody is then washed from the cells. Cells expressing CD90 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD90. Cell surface expression of CD90 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 mouse anti-human CD90/Thy-1: Use as is; no preparation necessary.



Human Jurkat T leukemic cells stained with APC-conjugated anti-Thy-1/CD90 (Catalog # FAB2067A, filled histogram) or isotype control (Catalog # IC003A 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 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 (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-CD90 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-CD90 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 APC-labeled mouse IgG2a antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

The human Thy-1 antigen/CD90 is an 18 kDa, GPI-linked, cell surface glycoprotein (1) that is highly homologous to members of the Ig superfamily (1, 2). Expression of CD90 in the brain is conserved across species and, as such, CD90 is believed to play an important role in the development and/or function of the nervous system (3, 4). Human CD90 is also expressed on a subset of $CD34^+$ hematopoietic stem cells capable of long-term growth in culture (5). In addition, CD90 is expressed on a variety of stromal and fibroblast cell lines (4, 5), activated endothlium (6) and tumor cell lines of neuronal and lymphoid origin (1, 7, 8). The ligand(s) for CD90 includes the integrin Mac-1 (CD11b/CD18) on monocytes and PMNs (6) indicating that CD90 may play a role in leukocyte adhesion and migration (6).

References

1. Seki, T. *et al.* (1985) Proc. Natl. Acad. Sci. USA **82**:6657.
2. Williams, A.F. and J. Gagnon (1982) Science **216**:696.
3. Morris, R. (1985) Dev. Neurosci. **7**:133.
4. Barlow, J.Z. *et al.* (2002) Neuroscience **111**:837.
5. Craig, W. *et al.* (1993) J. Exp. Med. **177**:1331.
6. Wetzel, A. *et al.* (2004) J. Immunol. **172**:3850.
7. Tiveron, M.C. *et al.* (1992) Nature **355**:745.
8. Sugimoto, T. *et al.* (1988) Cancer Res. **48**:2531.

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.