Polyclonal Anti-mouse PD-1-Phycoerythrin

Catalog Number: FAB1021P
Lot Number: AAHD01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated goat polyclonal anti-mouse PD-1: Supplied as 50 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Isotype: goat IgG

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

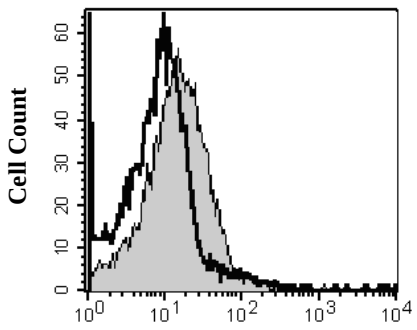
Designed to quantitatively determine the percentage of cells bearing PD-1 within a population and qualitatively determine the density of PD-1 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled polyclonal antibody, which binds to cells expressing PD-1. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing PD-1 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of PD-1. Cell surface expression of PD-1 is determined by flow cytometric analysis using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.

Reagent Preparation

Phycoerythrin-conjugated goat anti-mouse PD-1: Use as is; no preparation necessary.



Mouse CD4+ T-cells were stained with PE-conjugated anti-mouse PD-1 (Catalog # FAB1021P, filled histogram) or isotype control (Catalog # IC108P, 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 mouse 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 PE-conjugated PD-1 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted PD-1 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 PE-labeled goat IgG antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

Programmed Death-1 (PD-1) is type I transmembrane protein belonging to the CD28/CTLA-4 family of immunoreceptors that mediates signals for regulating immune responses.[1] Other members of this family include CD28, CTLA-4 and ICOS.[2 - 4] PD-1 is most closely related to CTLA-4 and shares approximately 24% amino acid (aa) sequence identity. The mouse PD-1 gene encodes a 288 aa protein with a putative 20 aa signal peptide, a 149 aa extracellular region with one immunoglobulin-like V-type domain, a 21 aa transmembrane domain, and a 98 aa cytoplasmic region. The cytoplasmic tail contains two tyrosine residues that form the immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM) that are important in mediating PD-1 signaling. Mouse and human PD-1 share approximately 69% aa sequence identity. Two B7 family proteins, PD-L1 (also called B7-H1) and PD-L2, have been identified as PD-1 ligands.[5, 6] PD-1 is expressed on activated T-cells, B-cells, myeloid cells and on a subset of thymocytes. Mice deficient in PD-1 have a defect in peripheral tolerance and spontaneously develop autoimmune diseases. Binding of PD-1 to PD-L1 or PD-L2 results in the inhibition of TCR-mediated proliferation and cytokine production as well as BCR-mediated signaling. PD-1 likely has an inhibitory role in regulating immune responses.[1 - 4]

References

1. Ishida, Y. *et al*., 1992, EMBO J. **11**:3887 - 3895.
2. Sharpe, A.H. and G. J. Freeman, 2002, Nat. Rev. Immunol. **2**:116.
3. Coyle, A. and J. Gutierrez-Ramos, 2001, Nat. Immunol. **2**:203.
4. Nishimura, H. and T. Honjo, 2001, Trends in Immunol. **22**:265.
5. Latchman Y. *et al*., 2001, Nature Immun. **2**:261.
6. Tamura, H. et al., 2001, Blood 97:1809.

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.