Monoclonal anti-human CD28-Phycoerythrin

Catalog Number: FAB342P

Reagents Provided

This kit provides enough reagents for a total of 100 reactions.

Clone #: 37407

Isotype: mouse IgG_1

Phycoerythrin-conjugated mouse monoclonal anti-human CD28: contains 1.0 mL of phycoerythrin-labeled antibody [50 µg/mL].

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Storage

All Reagents: 2 - 8° C

Intended Use

Designed to quantitatively determine the percentage of cells bearing CD28 within a population and qualitatively determine the density of CD28 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to the cells expressing CD28. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing the CD28 structure are fluorescently stained, with the intensity of staining directly proportional to the density of expressed CD28. Cell surface expression of CD28 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Phycoerythrin-conjugated mouse anti-human CD28: Use as is; no preparation necessary.

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Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anti-coagulant. 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 4×10^6 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 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 (1×10^5 cells) or 50 µL of packed whole blood to a 5 mL tube.
3) Add 10 µL of PE-conjugated anti-CD28 reagent.
4) Incubate for 30-45 minutes at 2 - 8° C.
5) Following this incubation, remove unreacted anti-CD28 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, Cat # 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 murine IgG_1 antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

The acceptance of the two signal model of T-cell activation originally proposed by Bretscher and Cohn (1) provided the impetus to elucidate the various molecules involved in the T cell activation cascade. CD28, a 44 kDa homodimeric glycoprotein, is one member of these T cell activation molecules (2). CD28 is the receptor for a group of molecules referred to as the family of B7 antigens (3,4). Interaction of antigen presenting cell expressed molecules like B7-1 (CD80) and B7-2 (CD86) with CD28 regulate the type of T cell response. Although CD80 and CD86 have a complex role in T cell activation, the working hypothesis is that CD28-CD86 interactions may be required for initial T cell costimulation, while CD28-CD80 interactions appear involved in sustaining T cell activation.

CD28 is a type I transmembrane protein with a single immunoglobulin-like domain. Human CD28 shares 78% sequence homology with mouse CD28 (5). CD28 has been reported to be expressed on thymocytes, T cells, plasma cells and gamma/delta T cells (6,7,8). The action of CD28 is further tempered by the fact that its two ligands, CD80 and CD86, can also interact with another T cell expressed receptor CTLA-4 (CD152) which delivers a negative activation signal (9). Other members of the CD28 receptor family include ICOS (10) and PD-1 (11). In addition to B7-1 and B7-2, this family of molecules now contains new members like B7RP-1, PD-L1, PD-L2 and B7-H3 (4). These ligands in concert with their respective receptors play a complex role in regulating T cell activation.

References

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Note: 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.