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Product Information

Anti-CD28 antibody, Mouse monoclonal
clone CD28.2, purified from hybridoma cell culture

Product Number **C7831**

Product Description

Anti-CD28 antibody, Mouse monoclonal (mouse IgG1 isotype) is derived from the CD28.2 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Human CD28 transfected murine T- cell hybridoma was used as the immunogen.[1] The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2.

Monoclonal Anti-Human CD28 recognizes the human CD28 antigen expressed by most T lineage cells. The antibody reacts with human CD28-transfected murine T-cell hybridoma, PHA-activated blasts and IL-6 dependent plasmacytoma lines. It cross reacts with rhesus monkey PBL. The antibody blocks CD28/B7 (CD80) mediated cell-cell adhesion. It binds to the CDR domain in a CDR1 dependent way.[2]

Human CD28 antigen is a 44 kDa disulfide linked homodimeric T cell specific surface glycoprotein.[3,4] It is a cell adhesion molecule of the immunoglobulin superfamily which is constitutively expressed on most peripheral blood T lymphocytes (approximately 95% of $CD4^+$ cells and 50% of $CD8^+$ cells). Mature thymocytes exhibit higher levels of CD28 than the immature cells. Activation of T cells results in enhanced CD28 expression. T cell activation requires two combined signals provided by antigen presenting cells. The first is mediated via the T cell receptor following its interaction with antigenic peptide-MHC complexes, and the second is delivered by accessory or co-stimulating molecules through their counter-receptors on T lymphocytes. CD28 bears structural homology to CTLA-4 which is expressed at very low levels on the surface of $CD4^+$ and $CD8^+$ peripheral blood cells only following activation. CD28 is the natural receptor for the B7/BB-1 ligand (CD80)[5,6] a 55-60 kDa glycoprotein which is expressed on activated B lymphocytes, on dendritic cells and on interferon-γ treated monocytes. The binding of B7-1/BB-1 molecules to CD28 is involved in T lymphocyte activation and in the initiation and maintenance of chronic inflammation. CD28 provides co-signalling for proliferation and activation and induces PI3-kinase activity.[7,8,9] CD28 stimulation acts at both the transcriptional and post transcriptional levels (mRNA stability). CD28 binds also to B7-2/B70 (CD86) a 70 kDa cell surface glycoprotein which rapidly appears on B cells after activation.[10,11] B7-2 is constitutively expressed on monocytes but its expression is increased following interferon-γ treatment. It is also expressed on dendritic cells and, like B7-1, is induced to a low level in chronically stimulated T cells. The signal provided via CD28 seems necessary for induction of clonal expansion and prevention of T cell anergy. Monoclonal antibodies to CD28 may be comitogenic for T cells in the presence of submitogenic concentrations of CD3 monoclonal antibodies, CD2 monoclonal antibodies, PHA and phorbol esters. Costimulation of T cells by CD28 monoclonal antibodies combined with CD2 and CD3 monoclonal antibodies is mediated via the synthesis of large amount of cytokines from T cells (IL-2) or from accessory cells (IL-1α and TNF-α) as well as the induction of IL-2 receptor chains. Monoclonal Anti-Human CD28 (clone CD28.2) induces some IL-2 secretion in the presence of phorbol-12 myristate-13-acetate in human T leukemia Jurkat cells and induces a rapid and strong increase in intracellular calcium in these cells. It also enhances c-rel protein translocation in human peripheral blood mononuclear cells. The antibody is an efficient co-stimulator with CD2 antibodies or PHA. It generates a co-stimulatory signal for CD3 antibody induced T-cell proliferation that is resistant to inhibition by cyclosporin A.

Monoclonal Anti-Human CD28 may be used for:
1. Identification and enumeration of T lymphocytes in peripheral blood.
2. T cell isolation by sorting procedures.
3. Cell activation studies.
4. Cell adhesion studies.

Reagents

Supplied as purified antibody (100-200 μg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 15 mM sodium azide as a preservative.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Performance
When assayed by flow cytometric analysis, 5 μl of the monoclonal antibody will stain 1 x 10^6 cells with a fluorescence intensity and percent positive that is similar to that observed with saturating amounts of monoclonal antibody.

Storage
Store at 2-8 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure for Indirect Immunofluorescent Staining
Reagents and Materials Needed but Not Supplied

1. a. Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A or heparin anticoagulant **or**
   b. Human cell suspension (e.g. peripheral blood mononuclear cells isolated on Histopaque®, Catalog Niumber 10771).
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1%NaN_3.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Product No. F 2883 FITC-Sheep Anti-Mouse IgG (whole molecule), F(ab')$_2$ fragment of Affinity Isolated Antibody). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
4. Isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. M5284).
5. 12 x 75 mm test tubes.
6. Adjustable micropipet.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (Product No. T0776) in 0.01 M phosphate buffered saline, pH 7.4.
10. 2% paraformaldehyde in PBS.
11. Whole blood lysing solution.
12. Flow cytometer.

Procedure

1. a. Use 100 μl of whole blood **or**
   b. Adjust cell suspension to 1 x 10^7 cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (e.g., trypan blue). For each sample, add 100 μl or 1 x 10^6 cells per tube.
2. Add 5 μl of monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently. Incubate the cells at room temperature (18 – 22 °C) for 30 minutes. Proper controls to be included for each sample are:
   a. Autofluorescence control: 5 μl diluent in place of monoclonal antibody.
   b. Negative staining control: 5 μl isotype-matched non-specific mouse immunoglobulin (Product No. M 5284) at the same concentration as test antibody.
3. After 30 minutes, add 2 ml of diluent to all tubes.
4. Pellet cells by centrifugation at 500 x G, for 10 minutes.
5. Remove supernatant by careful aspiration.
6. Resuspend cells in 2 ml diluent.
7. Repeat washing procedure (steps 4-6).
8. After the second wash, resuspend the cells in 100 μl of the fluorochrome conjugated secondary - antibody at the recommended concentration. For the autofluorescence control, add 100 μl of diluent. Incubate at room temperature (18 – 22 °C) for 30 minutes. Protect from light at this and all subsequent steps.
9. a. If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then proceed to Step 10.
   b. If a mononuclear cell suspension is used, proceed to Step 10.
10. Add 2 ml diluent to all tubes.
11. Wash as in steps 4-6 twice.
12. After last wash, resuspend cells in 0.5 ml of 2% paraformaldehyde and analyze in a flow cytometer according to manufacturer's instructions.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein. It should be isotype-matched to the primary antibody, not specific for human cells and of the same concentration as the primary antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used.

For fluorescence analysis of cells with Fc receptors, the use of isotype matched negative control is mandatory. In some systems it may be necessary to incubate the cells in 10-20% normal serum from the second antibody host species (at step 2 before adding monoclonal antibody) in order to decrease non-specific staining with the conjugated second antibody.

Selected References

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