**Product No. F-2792**
**Lot 105H4835**

**Monoclonal Anti-Human CD28**
**FITC Conjugate**
Purified Mouse Immunoglobulin
Clone CD28.2

Monoclonal Anti-Human CD28 (mouse IgG1 isotype) is derived from the CD28.2 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with the human CD28 transfected murine T cell hybridoma.[1] The isotype is determined using Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I with purified CD28 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable. The antibody is provided (100 µg/ml) as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide (see MSDS)* as a preservative.

**Description**

FITC 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 kD 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 kD 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 kD 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.

**Performance**

When assayed by flow cytometric analysis, using 10 µl of the antibody to stain 1 x $10^6$, cells a fluorescence intensity is observed similar to that obtained with saturating monoclonal antibody levels. The percent population positive is also at the maximum percentage positive using saturating monoclonal antibody levels.

**F/P Molar Ratio:** 5.2

**Uses**

FITC Conjugated 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.

**Storage**

Store at 2-8°C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

* Due to the sodium azide content a material safety sheet (MSDS) has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Note: In order to obtain best results in different preparations it is recommended that each individual user determine their optimum working dilutions by titration assay.

**Procedure for Direct 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® (Sigma Product No. 1077-1)).
2. Diluent: 0.01 M Phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% $NaN_3$.
3. FITC conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Sigma Product No. F-6397).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Sigma Product No. T-0776), 0.2% in 0.01 M PBS, pH 7.4.
9. 2% paraformaldehyde in PBS.
10. Whole blood lysing solution.
11. 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 10 µl of conjugate 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. An autofluorescence control: 10 µl diluent in place of monoclonal antibody, followed by steps 3 - 7.
   b. A negative staining control: 10 µl of FITC conjugated, isotype-matched non-specific mouse immunoglobulin (Sigma Product No. F-6397) at the same concentration as test antibody followed by steps 3 - 7.
3. a. If whole blood is used, use lysing solution after incubation and wash cells according to manufacturer's instructions.
   b. If a mononuclear cell suspension is used, proceed to Step. 4.
4. Add 2 ml of diluent to all tubes.
5. Pellet cells by centrifugation at 500 x G for 10 minutes.
6. Remove supernatant by careful aspiration.
7. Resuspend cells in 0.5 ml of 2% paraformaldehyde. 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 binding of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein which has no reactivity with human cells. It should be isotype-matched to the antibody and of the same concentration and F/P molar ratio as the 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.

**References**


1. Nunès J., et al., Intern. Immunol., **5**, 311 (1993).
2. Leucocyte Typing V, Oxford University Press, No. CD28.05, p 360 (1995).
3. Hansen, J., et al., Immunogenetics, **10**, 247 (1980).
4. Linsley, P., et al., Proc. Natl. Acad. Sci. USA, **87**, 5031 (1990).
5. Yokochi, T., et al., J. Immunol., **128**, 823 (1982).
6. Freeman, G., et al., J. Immunol., **143**, 2714 (1989).
7. Linsley, P., et al., Ann. Rev. Immunol., **11**, 191 (1993).
8. Janeway, C., et al., Cell, **76**, 275 (1994).
9. Pagès, F., et al., Nature, **369**, 327 (1994).
10. Azuma, M., et al., Nature, **366**, 76 (1993).
11. Freeman, G., et al., Science, **262**, 909 (1993).