

## Product Information

### Monoclonal Anti-Human CD28 R-Phycoerythrin Conjugate Purified Mouse Immunoglobulin Clone CD28.2

Product No. **P 3213**

#### Product Description

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 human CD28 transfected murine T cell hybridoma.<sup>1</sup> The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The product is protein A purified Monoclonal Anti-Human CD28 conjugated to R-Phycoerythrin (PE). The conjugate is purified by gel filtration to remove free PE and antibody.

PE 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. T cell activation studies
4. Simultaneous multicolor analysis when used in conjunction with FITC or Quantum Red™ conjugates.

PE 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 blasts and IL-6 dependent plasmacytoma lines. It cross reacts with Rhesus monkey PBL.<sup>2</sup> Human CD28 antigen is a 44 kDa disulfide linked homodimeric T cell specific surface glycoprotein.<sup>3,4</sup> It is a cell adhesion molecule of the immunoglobulin superfamily which is constitutively expressed on most peripheral blood T lymphocytes (approximately 95% of CD4<sup>+</sup> cells and 50% of CD8<sup>+</sup> 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<sup>+</sup> and CD8<sup>+</sup> peripheral blood cells only following activation. CD28 is the natural receptor for the B7/BB-1 ligand (CD80)<sup>5,6</sup> a 55-60 kDa glycoprotein which is expressed on activated B lymphocytes, on dendritic cells and on interferon- $\gamma$  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.<sup>7,8,9</sup> 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.<sup>10,11</sup> B7-2 is constitutively expressed on monocytes but its expression is increased following interferon- $\gamma$  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. Co-stimulation 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-1 $\alpha$  and TNF- $\alpha$ ) 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.

## Reagents

The product is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide as a preservative.

## Precautions and Disclaimer

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.

## Performance

When assayed by flow cytometric analysis, using 10  $\mu$ l of the conjugate to stain  $1 \times 10^6$  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. In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

$A_{567}/A_{280}$ : 2.8

## 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.

## Procedure for Direct Immunofluorescent Staining

### Reagents and Materials Needed but Not Supplied

- Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A or heparin anticoagulant or
  - Human cell suspension (e.g., peripheral blood mononuclear cells isolated on HISTOPAQUE (Product No. 1077-1)).
- Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1%  $\text{NaN}_3$ .
- PE conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. P 4685).
- 12 x 75 mm test tubes.
- Adjustable micropipet.
- Centrifuge.
- Counting chamber.
- Trypan blue (Product No. T 0776), 0.2% in 0.01 M PBS, pH 7.4.
- 2% paraformaldehyde in PBS.
- Whole blood lysing solution.
- Flow cytometer.

## Procedure

- Use 100  $\mu$ l of whole blood or
  - Adjust cell suspension to  $1 \times 10^7$  cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (e.g., trypan blue). For each sample, add 100  $\mu$ l or  $1 \times 10^6$  cells per tube.
- Add 10  $\mu$ l 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:
  - An autofluorescence control: 10  $\mu$ l diluent in place of monoclonal antibody, followed by steps 3 - 7.
  - A negative staining control: 10  $\mu$ l of PE conjugated, isotype-matched non-specific mouse immunoglobulin (Product No. P 4685) at the same concentration as test antibody followed by steps 3 - 7.
- If whole blood is used, use lysing solution after incubation and wash cells according to manufacturer's instructions.
  - If a mononuclear cell suspension is used, proceed to Step. 4.
- Add 2 ml of diluent to all tubes.
- Pellet cells by centrifugation at 500 x G for 10 minutes.
- Remove supernatant by careful aspiration.
- 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

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