

### Analyte Specific Reagent.

Analytical and performance characteristics are not established.

#### SPECIFICITY

The CD28 antigen, (Tp44), is a homodimeric, disulfide-linked, type 1 transmembrane protein; the monomer is 202 amino acid-long, with a molecular weight of 44 kDa (1, 2). The extracellular region, homologous to an Ig V-like domain, shares significant amino acid sequence with the CD152 antigen (CTLA-4) (3).

The CD28 antigen is involved in the interaction of T lymphocytes with professional antigen-presenting cells (APCs), through its counter-receptors, B7-1/BB-1 (CD80) and B7-2/B70 (CD86). It provides a major co-stimulatory signal for T cell activation, proliferation and lymphokine production. The CD28 family of receptors (CD28, CTLA-4, ICOS, PD-1 and BTLA) plays a critical role in controlling the adaptive immune response. The CD28 receptor can enhance T cell antigen receptor (TCR) signals, as well as deliver independent signals. Although the signals through CD28 are crucial for the initial co-stimulation of interleukin-2 (IL-2) production (4), a TCR-independent CD28 signal leads to the selective transcription of survival, but not proliferative genes (5).

The cytoplasmic region of CD28 can associate with the PI3-kinase (2), the GRB-2/SOS complex, and the T cell-specific protein-tyrosine kinase ITK (6, 7). The pYMM motif of the intracellular domain of CD28 binds to the SH2 domains of PI3-kinase and GRB-2.

CD28 is expressed on T cells, on plasma cells and thymocytes (1, 8).

The CD28.2 monoclonal antibody has been assigned to the CD28 cluster of differentiation at the 5th International Workshop on Human Leucocyte Differentiation Antigens in Boston, U.S.A., in 1993 (1).

#### REAGENT

IOTest CD28-PE Conjugated Antibody  
PN IM2071U – 2 mL Liquid – 20 µL / test\*.

<b>Clone</b>	CD28.2
<b>Isotype</b>	IgG1, mouse
<b>Immunogen</b>	Transfected murine cell line
<b>Hybridoma</b>	X63 Ag8.653 X Balb/c
<b>Source</b>	Ascites fluid
<b>Purification</b>	Ion exchange or affinity chromatography
<b>Conjugation</b>	R-phycoerythrin (PE) is conjugated at 0.5 – 1.5 moles of PE per mole of Ig.
<b>Fluorescence</b>	PE (orange-red) Excites at 486 – 580 nm Emits at 568 – 590 nm

#### REAGENT CONTENTS

This reagent is provided in phosphate-buffered saline, with 0.1% sodium azide

(NaN<sub>3</sub>) as preservative, and 2.0 mg / mL bovine serum albumin (BSA).

#### STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Do not use antibody beyond the expiration date on the label.
3. Samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
4. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
5. Minimize exposure of reagent to light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.
7. Use good laboratory practices when handling this reagent.

#### STORAGE CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze. Minimize exposure to light.

#### EVIDENCE OF DETERIORATION

Any change in the physical appearance of this PE-labeled reagent (clear colorless to pinkish liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.

#### REAGENT PREPARATION

No preparation is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

#### SELECTED RESEARCH REFERENCES

1. Olive, D., Cerdan, C., Costello, R., Sielleur, I., Ragueneau, M., Pages, F., Klasen, S., Nunès, J., Imbert, J., "CD28 and CTLA-4 cluster report", 1995, in: *Leucocyte Typing V, White Cell Differentiation Antigens*, Vol. 1. Schlossman, S.F., et al., Eds., Oxford University Press, 360-370.
2. Ghiotto-Ragueneau, M., Battifora, M., Truneh, A., Waterfield, M.D., Olive, D., "Comparison of CD28-B7.1 and B7.2 functional interaction in resting human T cells: phosphatidylinositol 3-kinase association to CD28 and cytokine production", 1996, *Eur. J. Immunol.*, 26, 34-41.

3. Buonavista, N., "Molecular linkage of the human CTLA4 and CD28 Ig-superfamily genes in yeast artificial chromosomes", 1992, *Genomics*, 13, 856-861.
4. Sharpe, A.H., Freeman, G.J., "The B7-CD28 superfamily", 2002, *Nature Rev. Immunol.*, 2, 116-126.
5. Marinari, B., Costanzo, A., Marzano, V., Piccolella, E., Tuosto, L., "CD28 delivers a unique signal leading to the selective recruitment of RelA and p52 NF-κB subunits on IL-8 and Bcl-xL gene promoters", 2004, *Proc. Natl. Acad. Sci. USA*, 101, 6098-6103.
6. June, C.H., Bluestone, J.A., Nadler, L.M., Thompson, C.B., "The B7 and CD28 receptor families", 1994, *Immunol. Today*, 15, 321-31.
7. Raab, M., Cai, Y., Bunnell, S.C., Heyeck, S.D., Berg, L.J., Rudd, C.E., "p<sup>56</sup>Lck and p<sup>59</sup>Fyn regulate CD28 binding to phosphatidylinositol 3-kinase, growth factor receptor-bound protein GRB-2, and T cell-specific protein-tyrosine kinase ITK: implications for T-cell costimulation", 1994, *Proc. Natl. Acad. Sci. USA*, 92, 8891-8895.
8. Bani, L., David, D., Moreau, J.L., Cayota, A., Nakarai, T., Ritz, J., Thèze, J., "Expression of the IL-2 receptor γ subunit in resting human CD4 T lymphocytes: mRNA is constitutively transcribed and the protein stored as an intracellular component", 1997, *Int. Immunol.*, 4, 9, 573-580.

#### PRODUCT AVAILABILITY

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PN IM2071U – 2 mL Liquid – 20 µL / test\*.

PE is licensed under patent 4,520,110.

For additional information in the USA, call 800-526-7694.  
Outside the USA, contact your local Beckman Coulter representative.

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(\*) : 20 µL is the quantity of product sufficient to stain

5 x 10<sup>5</sup> cells in a standard immunofluorescence assay