

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CD28 in direct ELISA.
Source	Recombinant Monoclonal Rabbit IgG Clone # 2946A
Purification	Protein A or G purified from cell culture supernatant
Immunogen	<i>S. frugiperda</i> insect ovarian cell line Sf21-derived recombinant human CD28 Asn19-Pro152 Accession # P10747
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Flow Cytometry	0.25 µg/10 ⁶ cells	PBMC lymphocytes with CD3 costain, and HEK293 cells transfected with Human CD28 and Comet vs irrelevant

DATA

Flow Cytometry

Detection of CD28 in PBMC lymphocytes cells by Flow Cytometry. PBMC lymphocytes were stained with Mouse Anti-Human CD3ε PE-conjugated Monoclonal Antibody (Catalog # FAB100P) and either (A) Rabbit Anti-Human CD28 Monoclonal Antibody (Catalog # MAB3422) or (B) Normal Rabbit IgG Control (Catalog # AB-105-C) followed by Allophycocyanin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0111). View our protocol for *Staining Membrane-associated Proteins*.

Flow Cytometry

Detection of CD28 in HEK293 cells transfected with Human CD28 and Comet vs irrelevant by Flow Cytometry HEK293 cells transfected with Human CD28 and Comet (A) vs irrelevant (B) were stained with Rabbit Anti-Human CD28 Monoclonal Antibody (Catalog # MAB3422) and isotype control antibody (Catalog # AB-105-C) followed by Allophycocyanin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0111). View our protocol for *Staining Membrane-associated Proteins*.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

CD28 and CTLA-4, together with their ligands B7-1 and B7-2, constitute one of the dominant costimulatory pathways that regulate T and B cell responses. CD28 and CTLA-4 are structurally homologous molecules that are members of the immunoglobulin (Ig) gene superfamily. Both CD28 and CTLA-4 are composed of a single Ig V-like extracellular domain, a transmembrane domain and an intracellular domain. CD28 and CTLA-4 are both expressed on the cell surface as disulfide-linked homodimers or as monomers. The genes encoding these two molecules are closely linked on human chromosome 2 and mouse chromosome 1. Mouse CD28 is expressed constitutively on virtually 100% of mouse T cells and on developing thymocytes. Cell surface expression of mouse CD28 is down-regulated upon ligation of CD28 in the presence of PMA or PHA. In contrast, CTLA-4 is not expressed constitutively but is up-regulated rapidly following T cell activation and CD28 ligation. Cell surface expression of CTLA-4 peaks approximately 48 hours after activation. Although both CTLA-4 and CD28 can bind to the same ligands, CTLA-4 binds to B7-1 and B7-2 with a 20-100 fold higher affinity than CD28. CD28/B7 interaction has been shown to prevent apoptosis of activated T cells via the up-regulation of Bcl-x_L. CD28 ligation has also been shown to regulate Th1/Th2 differentiation. Agonist activity has been reported using MAB4831 (4, 5).

References:

1. Lenschow, D.J. *et al.* (1996) *Annu. Rev. Immunol.* **14**:233.
2. Hathcock, K.S. and R.J. Hodes (1996) *Advances in Immunol.* **62**:131.
3. Ward, S.G. (1996) *Biochem. J.* **318**:361.
4. Nguyen, P. *et al.* (2003) *Blood* **13**:4320.
5. Orbach, A. *et al.* (2007) *J. Immunol.* **179**:7287.