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## CD28 (D2Z4E) Rabbit mAb

For Research Use Only. Not for Use in Diagnostic Procedures.

<b>Applications:</b> W, IP, IHC-Bond, IHC-P, IF-F	<b>Reactivity:</b> H M	<b>Sensitivity:</b> Endogenous	<b>MW (kDa):</b> 14, 40-60	<b>Source/Isotype:</b> Rabbit IgG	<b>UniProt ID:</b> #P10747	<b>Entrez-Gene Id:</b> 940
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### Product Usage Information

#### Application

Western Blotting  
Immunoprecipitation  
IHC Leica Bond  
Immunohistochemistry (Paraffin)  
Immunofluorescence (Frozen)

#### Dilution

1:1000  
1:50  
1:400 - 1:1600  
1:50 - 1:200  
1:50

### Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #87823.

### Specificity/Sensitivity

CD28 (D2Z4E) Rabbit mAb recognizes endogenous levels of total CD28 protein and is predicted to react with multiple CD28 isoforms that are produced through alternative splicing. This antibody cross-reacts with 16 kDa and 100 kDa proteins of unknown origin in some mouse cells. Non-specific staining was observed in mouse testis by immunohistochemistry.

### Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro208 of human CD28 protein.

### Background

CD28 is a transmembrane glycoprotein expressed by T cells as well as some other hematopoietic cells (1,2). T cell activation requires T cell receptor (TCR) recognition of antigen presented in the context of MHC molecules. CD28 acts as a T cell costimulatory receptor, and interaction of CD28 with its ligands CD80 or CD86 provides the second signal required for naive T cell activation (3-5). Activation of naive T cells in the absence of CD28 stimulation can result in a state of T cell anergy, or unresponsiveness (3). CD28 signals through cytoplasmic phospho-tyrosine motifs that bind several SH2 or SH3 domain-containing proteins involved in T cell activation (2). Recently, CD28 was demonstrated to be a preferred target of PD-1-mediated dephosphorylation. Consistently, CD28 expression was required for T cell proliferation following PD-1 blockade and CD28 stimulation was required for effective anti-PD-1 cancer immunotherapy in mice (6,7). Several CD28 isoforms are produced by alternative splicing (8).

### Background References

1. Aruffo, A. and Seed, B. (1987) *Proc Natl Acad Sci U S A* 84, 8573-7.
2. Esensten, J.H. et al. (2016) *Immunity* 44, 973-88.
3. Harding, F.A. et al. (1992) *Nature* 356, 607-9.
4. Azuma, M. et al. (1993) *Nature* 366, 76-9.
5. Linsley, P.S. et al. (1990) *Proc Natl Acad Sci U S A* 87, 5031-5.
6. Hui, E. et al. (2017) *Science* 355, 1428-1433.
7. Kamphorst, A.O. et al. (2017) *Science* 355, 1423-1427.
8. Magistrelli, G. et al. (1999) *Biochem Biophys Res Commun* 259, 34-7.

### Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

### Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

### Applications Key

**W:** Western Blotting **IP:** Immunoprecipitation **IHC-Bond:** IHC Leica Bond **IF-F:** Immunofluorescence (Frozen)

### Cross-Reactivity Key

**H:** Human **M:** Mouse **R:** Rat **Hm:** Hamster **Mk:** Monkey **Vir:** Virus **Mi:** Mink **C:** Chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** Zebrafish **B:** Bovine **Dg:** Dog **Pg:** Pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** Horse **GP:** Guinea Pig **Rab:** Rabbit **G:** Goat **All:** All Species Expected

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