Revision 1

Store at -20C

#16399

Phospho-CD28 (Tyr191) (E5B9Z) Rabbit mAb

Cell Signaling TECHNOLOGY®

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP	H M	Endogenous	40	Rabbit IgG	#P10747	940

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:200

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.*

Specificity/Sensitivity

Phospho-CD28 (Tyr191) (E5B9Z) Rabbit mAb recognizes endogenous levels of CD28 protein only when phosphorylated at Tyr191. This antibody shows cross-reactivity with phosphorylated EGFR, ERBB2, and CSF1R, and detects a 70-80 kDa band of unknown origin in some treated cell lines.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Tyr191 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 naïve T cell activation (3-5). Activation of naïve 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 nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **IP:** Immunoprecipitation

Cross-Reactivity Key

H: Human **M:** Mouse

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Limited Uses

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