Revision 5

Store at -20C

#11971

Phospho-SMAD1 (Ser463/465)/ SMAD5 (Ser463/465)/ SMAD9 (Ser465/467) (D5B10) Rabbit mAb (ChIP formulated)

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3 Trask Lane | Danvers | Massachusetts | 01923 | USA

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Applications:	Reactivity:	Sensitivity:	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
ChIP	H	Endogenous	Rabbit IgG	#Q99717, #Q15797, #O15198	4090, 4086, 4093

Product Usage Information

For optimal ChIP results, use 5 µl of antibody and 10 µg of chromatin (approximately 4 x 10^6 cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

Application	Dilution
Chromatin IP	1:100

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-SMAD1 (Ser463/465)/ SMAD5 (Ser463/465)/ SMAD9 (Ser465/467) (D5B10) Rabbit mAb (ChIP formulated) recognizes endogenous levels of SMAD1 and SMAD5 protein when phosphorylated at Ser463/465 and SMAD9 (SMAD8) protein when phosphorylated at Ser465/467.

Species predicted to react based on 100% sequence homology

Mouse, Rat, Monkey

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser463/465 of human SMAD1 and SMAD5 protein.

Background

Bone morphogenetic proteins (BMPs) constitute a large family of signaling molecules that regulate a wide range of critical processes including morphogenesis, cell-fate determination, proliferation, differentiation, and apoptosis (1,2). BMP receptors are members of the TGF-β superfamily of Ser/Thr kinase receptors. Ligand binding induces multimerization, autophosphorylation, and activation of these receptors (3-5). They subsequently phosphorylate SMAD1 at Ser463 and Ser465 in the carboxy-terminal motif SSXS, as well as SMAD5 and SMAD9 (SMAD8) at their corresponding sites. These phosphorylated SMADs dimerize with the coactivating SMAD4 and translocate to the nucleus, where they regulate the transcription of target genes (5). MAP kinases and CDKs 8 and 9 are also reported to phosphorylate residues in the linker region of SMAD1, including Ser206. Phosphorylation of SMAD1 at Ser206 recruits Smurf1 to the linker region and leads to the degradation of SMAD1 (6). Phosphorylation at this site also promotes SMAD1 transcriptional activity by recruiting YAP to the linker region (7).

Background References

1. Hogan, B.L. (1996) *Genes Dev* 10, 1580-94.
2. Hoodless, P.A. et al. (1996) *Cell* 85, 489-500.
3. Klemm, J.D. et al. (1998) *Annu Rev Immunol* 16, 569-92.
4. Kretzschmar, M. et al. (1997) *Genes Dev* 11, 984-95.
5. Whitman, M. (1998) *Genes Dev* 12, 2445-62.
6. Sapkota, G. et al. (2007) *Mol Cell* 25, 441-54.
7. Alarcón, C. et al. (2009) *Cell* 139, 757-69.

Species Reactivity

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

Applications Key

ChIP: Chromatin IP

Cross-Reactivity Key

H: Human

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Limited Uses

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