Revision 1

Store at -20C

#9511

Phospho-Smad1 (Ser463/465)/ Smad5 (Ser463/465)/ Smad9 (Ser465/467) Antibody

Cell Signaling TECHNOLOGY®

Orders: 877-616-CELL (2355) orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

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

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP, ChIP	H M R Mi	Endogenous	60	Rabbit	#Q99717, #Q15797, #O15198	4090, 4086, 4093

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100
Chromatin IP	1:50

Storage

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

Specificity/Sensitivity

Phospho-Smad1 (Ser463/465)/Smad5 (Ser463/465)/Smad9 (Ser465/467) Antibody detects endogenous levels of Smad1 only when phosphorylated at serine 463 and serine 465, as well as Smad5 and Smad9 (Smad8) only when phosphorylated at the equivalent sites. The antibody does not cross-react with other Smad-related proteins.

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser463/465 of human Smad5. Antibodies are purified by protein A and peptide affinity chromatography.

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).

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 **ChIP:** Chromatin IP

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat **Mi:** Mink

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Limited Uses

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