

Phospho-Smad1/5 (Ser463/465) (41D10) Rabbit mAb



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Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IF-IC, F Endogenous	H, M, R	60 kDa	Rabbit IgG**

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 family 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 Smad8 at their corresponding sites. These phosphorylated Smads dimerize with the coactivating Smad4 and translocate to the nucleus, where they stimulate transcription of target genes (5).

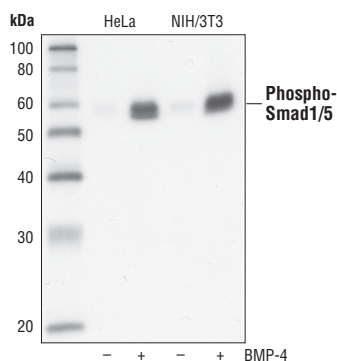
MAP kinases and CDKs 8 and 9 phosphorylate residues in the linker region of Smad1, including Ser206. The phosphorylation of Ser206 recruits Smurf1 to the linker region and leads to the degradation of Smad1 (6). Phosphorylation of this site also promotes Smad1 transcriptional action by recruiting YAP to the linker region (7).

Specificity/Sensitivity: Phospho-Smad1/5 (Ser463/465) (41D10) Rabbit mAb detects endogenous levels of Smad1 and Smad5 only when dually phosphorylated at Ser463 and Ser465 and is also predicted to detect Smad9 (Smad8) when phosphorylated at Ser465 and Ser467. The antibody does not cross-react with other Smad-related proteins.

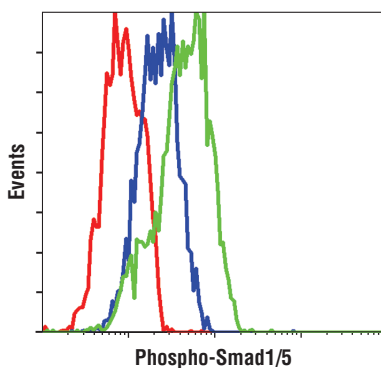
Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser463/465 of human Smad5.

Background References:

- Hogan, B.L. et al. (1996) *Genes Dev.* 10, 1580-1594.
- Hoodless, P.A. et al. (1996) *Cell* 85, 489-500.
- Klemm, J.D. et al. (1998) *Annu. Rev. Immunol.* 16, 569-592.
- Kretschmar, M. et al. (1997) *Genes Dev.* 11, 984-995.
- Whitman, M. (1998) *Genes Dev.* 12, 2445-2462.
- Sapkota, G. et al. (2007) *Mol Cell* 25, 441-54.
- Alarcón, C. et al. (2009) *Cell* 139, 757-69.



Western blot analysis of extracts from untreated or BMP-4-treated HeLa or NIH/3T3 cells using Phospho-Smad1/5 (Ser463/465) (41D10) Rabbit mAb.



Flow cytometric analysis of HeLa cells, untreated (blue) or BMP-treated (green), using Phospho-Smad1/5 (Ser463/465) (41D10) Rabbit mAb compared to a nonspecific negative control antibody (red).

Entrez-Gene ID #4090
UniProt Acc. #Q99717

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

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

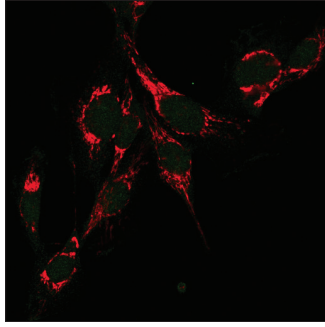
Western blotting	1:1000
Immunofluorescence (IF-IC)	1:800
IF Protocol:	Methanol Permeabilization required
Flow Cytometry	1:800

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

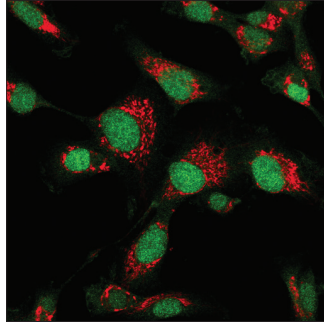
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

U.S. Patent No. 5,675,063
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Serum-starved



hBMP2-treated



Confocal immunofluorescent analysis of HT1080 cells, serum-starved (left) or serum-starved then treated with hBMP2 (50 ng/ml, 30 min; right) using Phospho-Smad1/5 (Ser463/465) (41D10) Rabbit mAb (green) and Cox IV (4D11-B3-E8) Mouse mAb #11967 (red).