Revision 10

Store at -20C

#9516

Phospho-SMAD1/5 (Ser463/465) (41D10) Rabbit mAb

Cell Signaling TECHNOLOGY®

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

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, W-S, IF-IC, FC-FP	H M R	Endogenous	60	Rabbit	#Q99717, #Q15797	4090, 4086

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50
Immunofluorescence (Immunocytochemistry)	1:800
Flow Cytometry (Fixed/Permeabilized)	1:400 - 1:1600

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 #52937.

Specificity/Sensitivity

Phospho-SMAD/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

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 nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **W-S:** Simple Western™ **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

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

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