#12534 Store at -20°C

Smad5 (D4G2) Rabbit mAb

☑ 100 µl
(10 western blots)

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New 03/13

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Entrez-Gene ID #4090
Swiss-Prot Acc. #Q99717

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, ChIP Endogenous	H, M, R, Mk	60 kDa	Rabbit IgG**

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.*

***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
Immunoprecipitation	1:50
Chromatin IP	1:100

For product specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended complementary products.

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: Smad5 (D4G2) Rabbit mAb recognizes endogenous levels of total Smad5 protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro249 of human Smad5 protein.

Background References:

(1) Hogan, B.L. et al. (1996) *Genes Dev.* 10, 1580-1594.

(2) Hoodless, P.A. et al. (1996) *Cell* 85, 489-500.

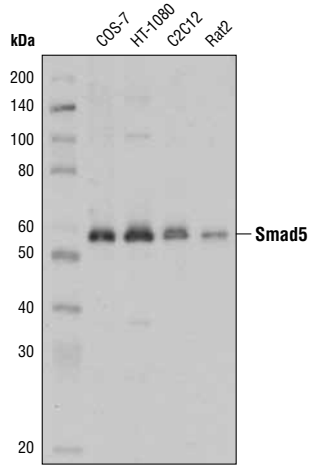
(3) Klemm, J.D. et al. (1998) *Annu. Rev. Immunol.* 16, 569-592.

(4) Kretzschmar, M. et al. (1997) *Genes Dev.* 11, 984-995.

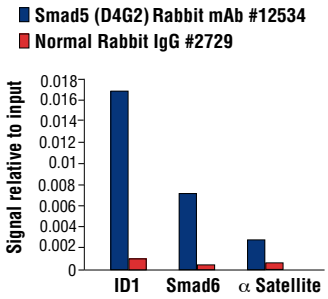
(5) Whitman, M. (1998) *Genes Dev.* 12, 2445-2462.

(6) Sapkota, G. et al. (2007) *Mol Cell* 25, 441-54.

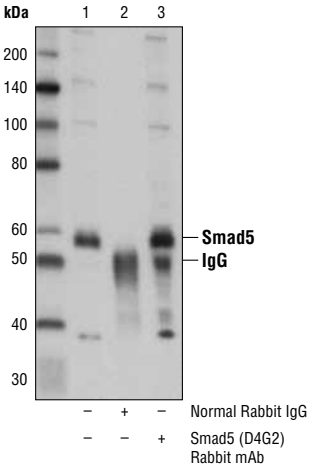
(7) Alarcón, C. et al. (2009) *Cell* 139, 757-69.



Western blot analysis of extracts from various cell lines using Smad5 (D4G2) Rabbit mAb.



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10⁶ MCF7 cells treated with Human BMP2 #4697 (50 ng/ml, 1 hr) and either 5 µl of Smad5 (D4G2) Rabbit mAb or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human ID1 Promoter Primers #5139, human Smad6 promoter primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.



Immunoprecipitation of Smad5 from HT-1080 cell extracts using Normal Rabbit IgG #2729 (lane 2) or Smad5 (D4G2) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using Smad5 (D4G2) Rabbit mAb.

IMPORTANT: For western blots, incubate membrane with diluted 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 **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.