#9511 Store at -20°C

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

Cell Signaling TECHNOLOGY®

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rev. 04/14/16

For Research Use Only. Not For Use In Diagnostic Procedures.

Entrez-Gene ID #4086
Swiss-Prot Acc. #Q15797

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

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.*

***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	1:100
Chromatin IP	1:50

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

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).

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 References:

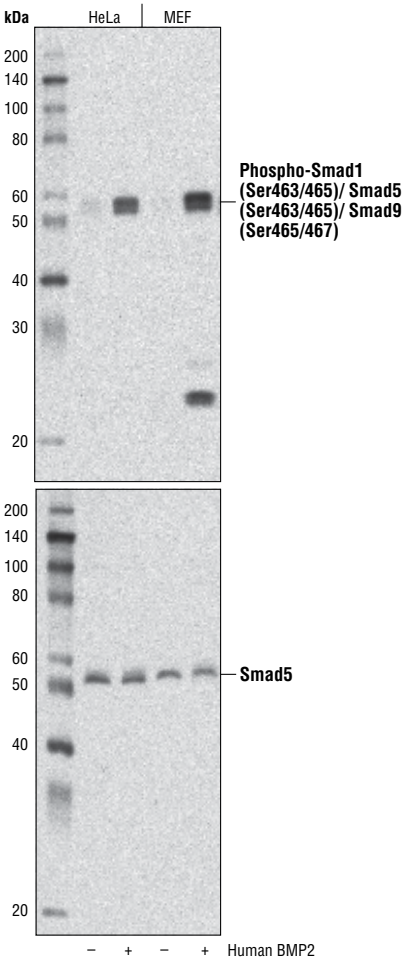
(1) Hogan, B.L. et al. (1996) *Genes Dev.* 10, 1580-1594.

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

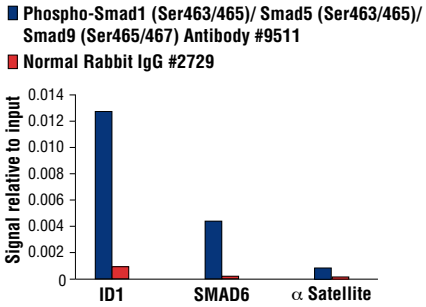
(3) Klemm, J.D. et al. (1998) *Annu. Rev. Immunol.* 16, 569-592.

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

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



Western blot analysis of extracts from HeLa or MEF cells, untreated (-) or treated with Human BMP2 #4697 (50 ng/ml, 30 min; +), using Phospho-Smad1 (Ser463/465)/ Smad5 (Ser463/465)/ Smad9 (Ser465/467) Antibody (upper) and Smad5 (D4G2) Rabbit mAb #12534 (lower).



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 MCF-7 cells treated with Human BMP2 #4697 (50 ng/ml) for 1 h and either 10 µl of Phospho-Smad1 (Ser463/465)/ Smad5 (Ser463/465)/ Smad9 (Ser465/467) Antibody 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.

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.

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.