

#9511 Store at -20°C

Phospho-Smad1 (Ser463/465)/ Smad5 (Ser463/465)/ Smad8 (Ser426/428) Antibody

- Small 100 µl
(10 western blots)
- Large 300 µl
(30 western blots)

rev. 05/06/11



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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

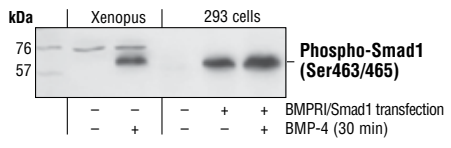
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, ChIP Endogenous	H, M, R, Mi, X	60 kDa	Rabbit**

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).

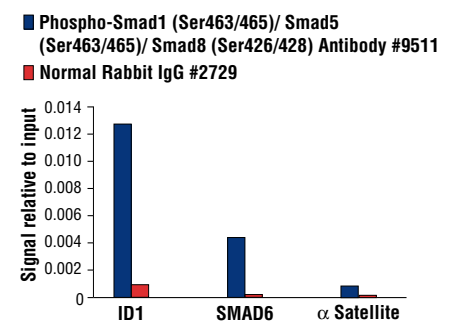
Specificity/Sensitivity: Phospho-Smad1 (Ser463/465)/Smad5 (Ser463/465)/Smad8 (Ser426/428) Antibody detects endogenous levels of Smad1 only when dually phosphorylated at serine 463 and serine 465, as well as Smad5 and Smad8 only when phosphorylated at the equivalent sites. The antibody does not cross-react 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:**
- 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.
 - Kretzschmar, M. et al. (1997) *Genes Dev.* 11, 984-995.
 - Whitman, M. (1998) *Genes Dev.* 12, 2445-2462.



Western blot analysis of extracts from untransfected *Xenopus* cells and BMPRI/Smad1-transfected 293 cells, untreated or BMP-4-treated, using Phospho-Smad1 (Ser463/465)/Smad5 (Ser463/465)/Smad8 (Ser426/428) Antibody. (*Xenopus* cells provided by Dr. Malcolm Whitman, Harvard University, Massachusetts.)



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 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)/Smad8 (Ser426/428) 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.

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

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 application 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 companion products.

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.

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