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Smad3 (C67H9) Rabbit mAb

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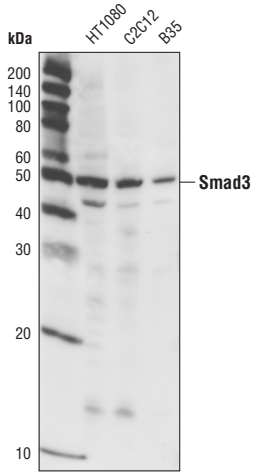
Entrez-Gene ID #4088
UniProt ID #P84022

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC, F, ChIP, ChIP-seq	H, M, R, Mk, (B, X, Z)	52 kDa	Rabbit IgG**

Background: Members of the Smad family of signal transduction molecules are components of a critical intracellular pathway that transmits TGF-β signals from the cell surface into the nucleus. Three distinct classes of Smads have been defined: the receptor-regulated Smads (R-Smads), which include Smad1, 2, 3, 5 and 8, the common-mediator Smad (co-Smad), Smad4, and the antagonistic or inhibitory Smads (I-Smads), Smad6 and 7 (1–5). Activated type I receptors associate with specific R-Smads and phosphorylate them on a conserved carboxy-terminal SSXS motif. The phosphorylated R-Smad dissociates from the receptor and forms a heteromeric complex with the co-Smad (Smad4), allowing translocation of the complex to the nucleus. Once in the nucleus, Smads can target a variety of DNA binding proteins to regulate transcriptional responses (6-8).

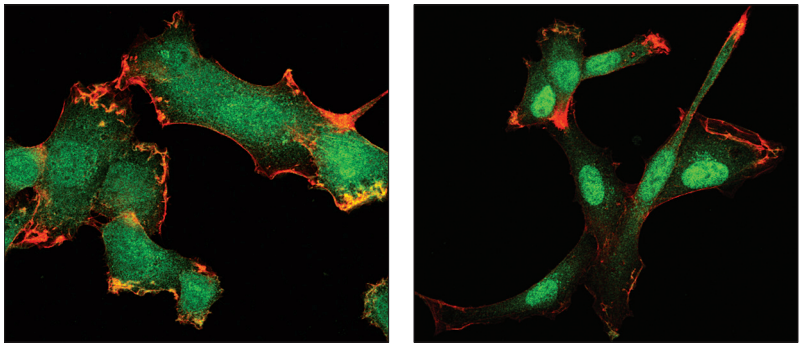
Following stimulation by TGF-β, Smad2 and Smad3 become phosphorylated at their carboxyl termini (Ser465 and 467 on Smad2; Ser423 and 425 on Smad3) by TGF-β Receptor I. Phosphorylated Smad 2/3 can complex with Smad4, translocate to the nucleus and regulate gene expression (9–11).

Specificity/Sensitivity: Smad3 (C67H9) Rabbit mAb detects endogenous levels of total Smad3 protein. No cross reactivity was detected with other family members.



Western blot analysis of extracts from HT1080 (human), C2C12 (mouse) and B35 (rat) using Smad3 (C67H9) Rabbit mAb.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues at the amino terminus of Smad3.



Confocal immunofluorescent analysis of HT1080 cells, untreated (left) or TGFβ-treated (right), using Smad3 (C67H9) Rabbit mAb (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red).

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.

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	1:100
Immunofluorescence (IF-IC)	1:100
Flow Cytometry	1:100
Chromatin IP / Chromatin IP-seq	1:50

Optimal ChIP/ChIP-seq conditions: 10 µl of antibody & 10 µg of chromatin (4 x 10^6 cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits.

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.

Background References:

(1) Heldin, C.H. et al. (1997) *Nature* 390, 465–471.
(2) Attisano, L. and Wrana, J.L. (1998) *Curr. Opin. Cell Biol.* 10, 188–194.
(3) Derynck, R. et al. (1998) *Cell* 95, 737–740.
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(5) Whitman, M. et al. (1998) *Genes Dev.* 12, 2445–2462.
(6) Wrana, J. (2000) *Science* 23, 1–9.
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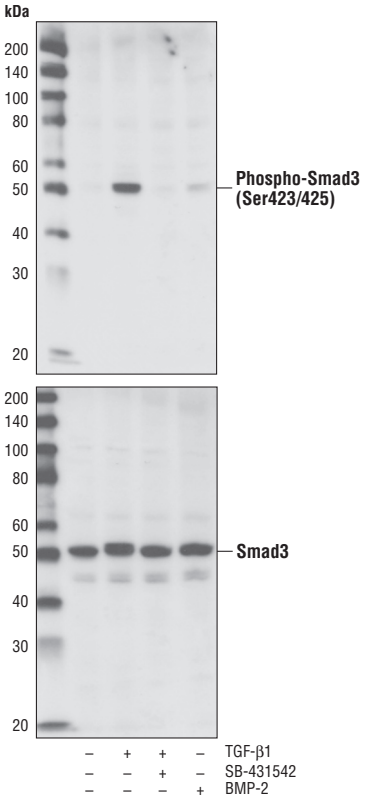
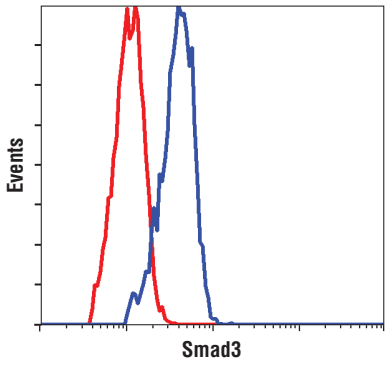
(11) Liu, X. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 10669–10674.

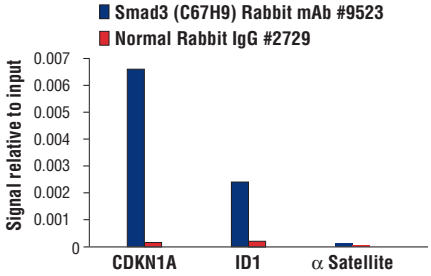
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **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.



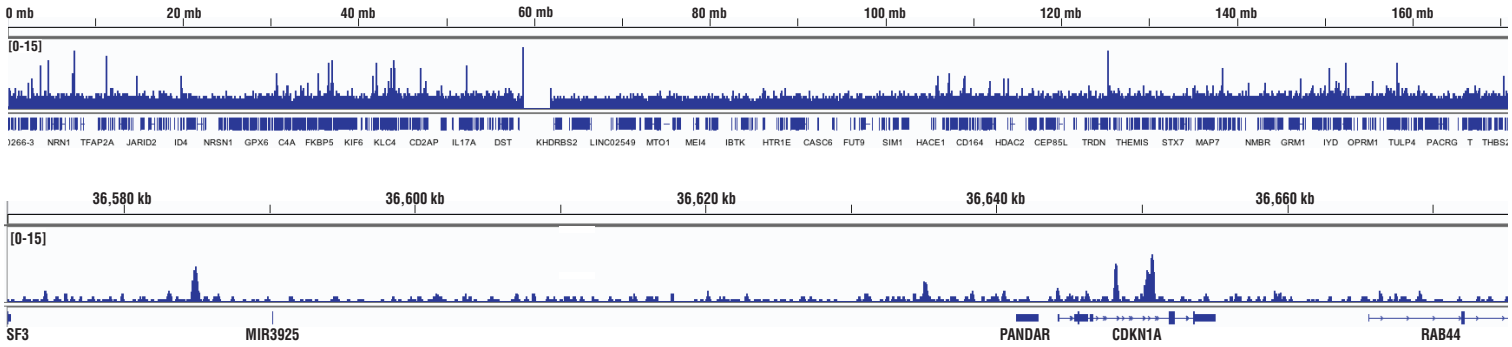
Western blot analysis of extracts from HT1080 cells, treated with TGF-β1, TGFR inhibitor SB-431542 or BMP-2, using Phospho-Smad3 (Ser423/425) (C25A9) Rabbit mAb #9520 (upper) or total Smad3 (C67H9) Rabbit mAb #9523 (lower).



Flow cytometric analysis of HT-1080 cells using Smad3 (C67H9) Rabbit mAb (blue) compared to a nonspecific negative control antibody (red).



Chromatin immunoprecipitations were performed with cross-linked chromatin from HaCaT cells treated with Human TGF-β3 #3706 (7 ng/ml) for 1 h and either Smad3 (C67H9) Rabbit mAb or Normal Rabbit IgG #2729 using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human CDKN1A Intron 1 Primers #4669, SimpleChIP® Human ID1 Promoter Primers #5139, 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.



Chromatin immunoprecipitations were performed with cross-linked chromatin from HaCaT cells treated with Human TGF-β3 #3706 (7 ng/ml) for 1 h and Smad3 (C67H9) Rabbit mAb, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. The figure shows binding across chromosome 6 (upper), including CDKN1A (lower), a known target gene of Smad3 (see additional figure containing ChIP-qPCR data).