Store at -20°C

#3108

Phospho-Smad2 (Ser465/467) (138D4) Rabbit mAb

☐ Small 100 µl
(10 western blots)

☐ Large 300 µl
(30 western blots)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 08/23/10

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

Entrez-Gene ID #4087
Swiss-Prot Acc. #Q15796

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

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

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

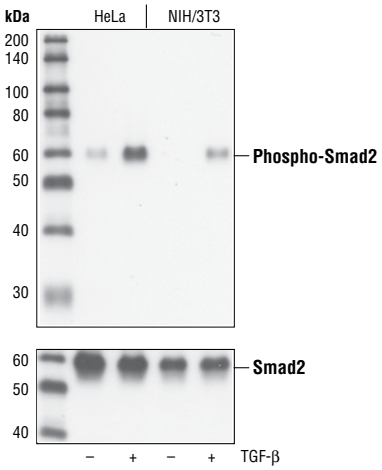
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background: 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 carboxy-termini (serines 465 and 467 on Smad2; serines 433 and 435 on Smad3) by the receptor kinase TGF-β-R1 (9-11). Following phosphorylation, Smad2 and Smad3 form a heteromeric complex with the co-smad family member Smad4. These complexes are translocated to the nucleus where they bind DNA and regulate gene transcription.

Specificity/Sensitivity: Phospho-Smad2 (Ser465/467) (138D4) Rabbit mAb detects endogenous levels of Smad2 only when dually phosphorylated at serines 465 and 467, and may detect Smad3 phosphorylated at the equivalent sites. This 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 Ser465/467 of human Smad2.



Western blot analysis of extracts from untreated or TGF-β treated HeLa and NIH/3T3 cells, using Phospho-Smad2 (Ser465/467) (138D4) Rabbit mAb (upper), or Smad2 Antibody #3102 (lower).

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.

(4) Massague, J. (1998) *Annu. Rev. Biochem.* 67, 753-791.

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

(6) Wu, G. et al. (2000) *Science* 287, 92-97.

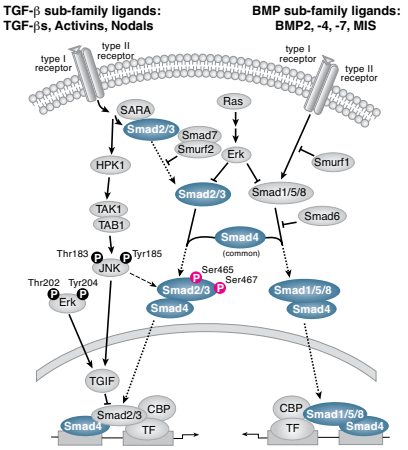
(7) Attisano, L. and Wrana, J.L. (2002) *Science* 296, 1646-1647.

(8) Moustakas, A. et al. (2001) *J. Cell Sci.* 114, 4359-4369.

(9) Abdollah, S. et al. (1997) *J. Biol. Chem.* 272, 27678-27685.

(10) Soucheinytskyi, S. et al. (1997) *J. Biol. Chem.* 272, 28107-28115.

(11) Liu, X. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 10669-10674.



Selected Rabbit Monoclonals are produced under license (granting certain rights, including those under U.S. Patent No. 5,675,063) from Epitomics, Inc.

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.

#5339 Store at -20°C

Smad2 (D43B4) XP® Rabbit mAb

☐ Small 100 µl
(10 western blots)

☐ Petite 40 µl
(4 western blots)

rev. 08/01/11

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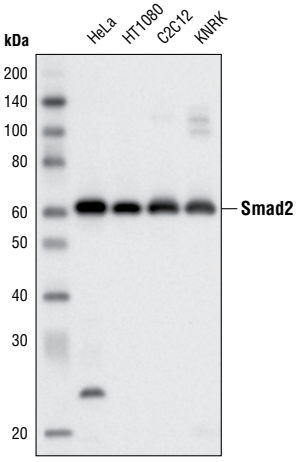
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC, F, ChIP Endogenous	H, M, R, Mk	60 kDa	Rabbit IgG**

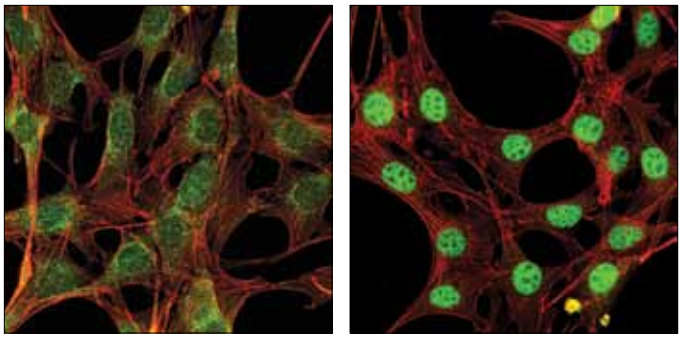
Background: Members of the Smad family of signal transduction molecules are components of a critical intracellular pathway that transmit TGF-β signals from the cell surface into the nucleus. Three distinct classes of Smads have been defined: the receptor-regulated Smads (R-Smads), which include Smad1, 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).

Specificity/Sensitivity: Smad2 (D43B4) XP® Rabbit mAb detects endogenous levels of total Smad2 protein. This antibody does not cross-react with Smad3.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of mouse Smad2 protein.



Western blot analysis of extracts from various cell lines using Smad2 (D43B4) XP® Rabbit mAb.



Confocal immunofluorescent analysis of NIH/3T3 cells, serum-starved (left) or treated with hTGF-β3 #8425 (right), using Smad2 (D43B4) XP® Rabbit mAb (green). Actin filaments have been labeled with DY-554 phalloidin (red).

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
Immunofluorescence (IF-IC)	1:100
Chromatin IP	1:50
Flow Cytometry	1:200

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

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Background References:

(1) Heldin, C.H. et al. (1997) *Nature* 390, 465-471.
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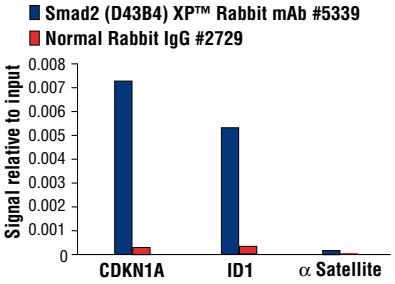
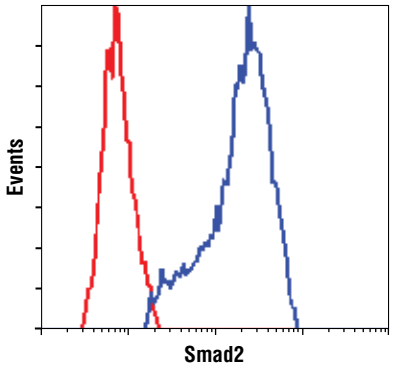


Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 HaCaT cells treated with Human TGF-β3 #8425 (7 ng/ml) for 1 h and either 10 µl of Smad2 (D43B4) XP® Rabbit mAb #5339 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP™ Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP™ Human 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.



Flow cytometric analysis of HeLa cells using Smad2 (D43B4) XP® Rabbit mAb (blue) compared to a nonspecific negative control antibody (red).