#8828 Store at -20°C

Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4) Rabbit mAb

Cell Signaling TECHNOLOGY®

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

rev. 03/08/16

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

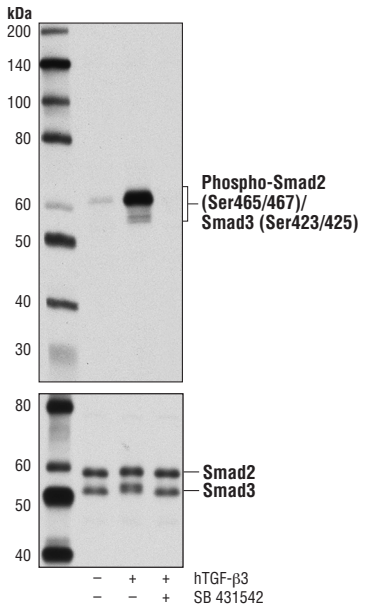
Entrez-Gene ID #4087, 4088
Swiss-Prot Acc. #Q15796, P84022

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IF-IC, F Endogenous	H, M, R, Mk	52, 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: Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4) Rabbit mAb recognizes endogenous levels of Smad2 protein when phosphorylated at Ser465 and Ser467. This antibody also recognizes endogenous levels of Smad3 protein when phosphorylated Ser422 only or at both Ser423 and Ser425.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser465/467 of human Smad2 protein.



Western blot analysis of extracts from HaCaT cells, untreated (-) or treated with hTGF-β3 #8425 (+) in the absence or presence of the TGFR inhibitor SB 431542, using Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4) Rabbit mAb (upper) or Smad2/3 (D7G7) XP® Rabbit mAb #8685 (lower).

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:

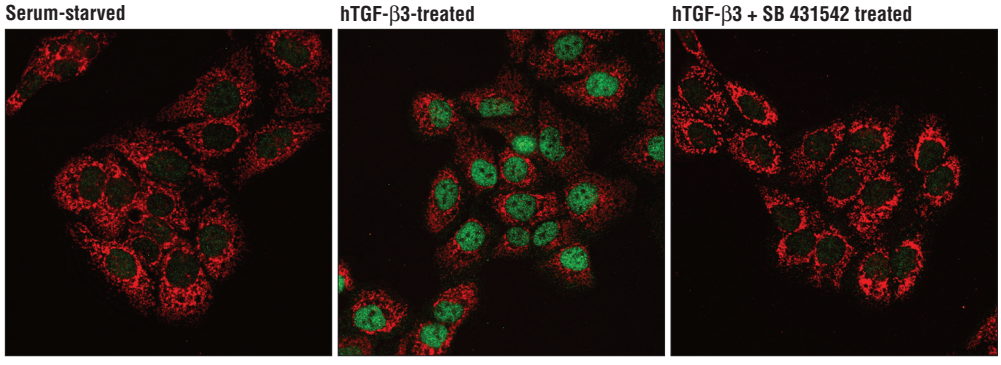
Western blotting	1:1000
Immunofluorescence (IF-IC)	1:200
IF Protocol:	Methanol Permeabilization required
Flow Cytometry	1:800

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 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.

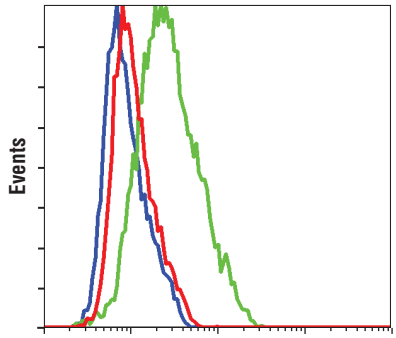


◀ *Confocal immunofluorescent analysis of HaCat cells, serum starved (left), treated with hTGF-β3 #8425 (100 ng/ml, 30 min; center), or treated with hTGF-β3 and SB 431542 (10 µg/mL, 1 hr; right), using Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4) Rabbit mAb (green) and COX IV (3E11) Rabbit mAb (Alexa Fluor® 555 Conjugate) #8693 (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.

Alexa Fluor® is a registered trademark of Molecular Probes, Inc.

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.



Flow cytometric analysis of HT-1080 cells, untreated (blue), treated with hTGF-β3 #8425 (green), or treated with hTGF-β3 and SB 431542 (red), using Phospho-Smad2 (Ser465/467)/ Smad3 (Ser423/425) (D27F4) Rabbit mAb.