

#9520 Store at -20°C

Phospho-Smad3 (Ser423/425) (C25A9) Rabbit mAb



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

rev. 01/31/18

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

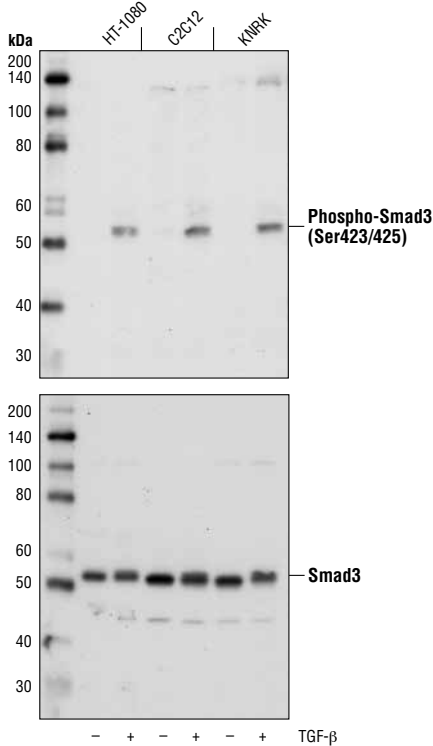
| Applications | Species Cross-Reactivity* | Molecular Wt. | Isotype |
|---------------------------|---------------------------|---------------|--------------|
| W, IP, ChIP Endogenous | H, M, R, (Mk, B, X, Z) | 52 kDa | Rabbit IgG** |

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).

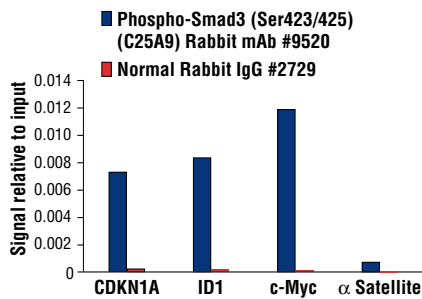
Following stimulation by TGF- β , Smad2 and Smad3 become phosphorylated at carboxyl terminal serine residues (Ser465 and 467 on Smad2; Ser423 and 425 on Smad3) by TGF- β Receptor I. Phosphorylated Smad 2/3 can complex with Smad4 and translocate to the nucleus to regulate gene expression (9–11).

Specificity/Sensitivity: Phospho-Smad3 (Ser423/425) (C25A9) Rabbit mAb detects endogenous levels of Smad3 when phosphorylated at Ser423/425. This antibody does not cross-react with other family members.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser423/425 of Smad3.



Western blot analysis of extracts from HT-1080, C2C12, or KNRK cells, untreated (-) or treated with TGF- β (10 ng/ml, 30 min; +), using Phospho-Smad3 (Ser423/425) (C25A9) Rabbit mAb (upper) or total Smad3 (C67H9) Rabbit mAb #9523 (lower).



◀ **Chromatin immunoprecipitations were performed with cross-linked chromatin from HaCaT cells treated with Human TGF- β 3 #3706 (7 ng/ml) for 1 h and either Phospho-Smad3 (Ser423/425) (C25A9) Rabbit mAb or 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, human c-Myc intron 1 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 #4088
UniProt ID #P84022

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/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 |
| Chromatin IP | 1:100 |

Optimal ChIP conditions: 5 μ l of antibody & 10 μ g of chromatin (4 x 10⁶ 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.
- (4) Massague, J. (1998) *Annu. Rev. Biochem.* 67, 753–791.
- (5) Whitman, M. et al. (1998) *Genes Dev.* 12, 2445–2462.
- (6) Wrana, J. (2000) *Science* 23, 1–9.
- (7) Attisano, L. and Wrana, J. (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) Souhelnitskyi, S. et al. (1997) *J. Biol. Chem.* 272, 28107–28115.
- (11) Liu, X. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 10669–10674.

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.

Tween20 is a registered trademark of ICI Americas, 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.