# #9523 Store at -20C

# SMAD3 (C67H9) Rabbit mAb



Orders: 877-616-CELL (2355)

orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com

cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP, IF-IC, FC-FP, ChIP, ChIP-seq	HMRMk	Endogenous	52	Rabbit IgG	#P84022	4088

# Product Usage Information

For optimal ChIP and ChIP-seq results, use 10  $\mu$ I of antibody and 10  $\mu$ g of chromatin (approximately 4 x 10<sup>6</sup> cells) per IP. This antibody has been validated using SimpleChIP<sup>®</sup> Enzymatic Chromatin IP Kits.

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (Immunocytochemistry)	1:100 - 1:200
Flow Cytometry (Fixed/Permeabilized)	1:100
Chromatin IP	1:50
Chromatin IP-seq	1:50

**Storage** 

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at  $-20^{\circ}$ C. Do not aliquot the antibody.

Specificity / Sensitivity

SMAD3 (C67H9) Rabbit mAb detects endogenous levels of total SMAD3 protein. No cross reactivity was detected with other family members.

Species predicted to react based on 100% sequence homology:

Xenopus, Zebrafish, Bovine

### Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues at the amino terminus of SMAD3.

## Background

Members of the SMAD family of signal transduction molecules are components of a critical intracellular pathway that transmit  $TGF-\beta$  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 9; 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-SMADs dissociate from the receptor and form a heteromeric complex with SMAD4, initiating translocation of the heteromeric SMAD complex to the nucleus. Once in the nucleus, SMADs recruit a variety of DNA binding proteins that function to regulate transcriptional activity (6-8).

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

### **Background References**

- 1. Heldin, C.H. et al. (1997) Nature 390, 465-71.
- 2. Attisano, L. and Wrana, J.L. (1998) Curr Opin Cell Biol 10, 188-94.
- 3. Derynck, R. et al. (1998) Cell 95, 737-40.
- 4. Massagué, J. (1998) Annu Rev Biochem 67, 753-91.
- 5. Whitman, M. (1998) Genes Dev 12, 2445-62.
- 6. Wrana, J.L. (2000) Sci STKE 2000, re1.
- 7. Attisano, L. and Wrana, J.L. (2002) Science 296, 1646-7.
- 8. Moustakas, A. et al. (2001) J Cell Sci 114, 4359-69.
- 9. Abdollah, S. et al. (1997) J. Biol. Chem. 272, 27678-27685.
- 10. Souchelnytskyi, S. et al. (1997) J. Biol. Chem. 272, 28107-28115.
- 11. Liu, X. et al. (1997) Proc. Natl. Acad. Sci. USA 94, 10669-10674.

### **Species Reactivity**

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

### **Western Blot Buffer**

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

# **Applications Key**

**WB**: Western Blotting **IP**: Immunoprecipitation **IF-IC**: Immunofluorescence (Immunocytochemistry) **FC-FP**: Flow Cytometry (Fixed/Permeabilized) **ChIP**: Chromatin IP **ChIP-seq**: Chromatin IP-seq

### **Cross-Reactivity Key**

H: human M: mouse R: rat Hm: hamster Mk: monkey Vir: virus 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 GP: Guinea Pig Rab: rabbit All: all species expected

# Trademarks and Patents

Cell Signaling Technology is a trademark of Cell Signaling Technology, Inc. Alexa Fluor is a registered trademark of Life Technologies Corporation.

U.S. Patent No. 7,429,487, foreign equivalents, and child patents deriving therefrom.

All other trademarks are the property of their respective owners. Visit cellsignal.com/trademarks for more information.

### **Limited Uses**

Except as otherwise expressly agreed in a writing signed by a legally authorized representative of CST, the following terms apply to Products provided by CST, its affiliates or its distributors. Any Customer's terms and conditions that are in addition to, or different from, those contained herein, unless separately accepted in writing by a legally authorized representative of CST, are rejected and are of no force or effect.

Products are labeled with For Research Use Only or a similar labeling statement and have not been approved, cleared, or licensed by the FDA or other regulatory foreign or domestic entity, for any purpose. Customer shall not use any Product for any diagnostic or therapeutic purpose, or otherwise in any manner that conflicts with its labeling statement. Products sold or licensed by CST are provided for Customer as the end-user and solely for research and development uses. Any use of Product for diagnostic, prophylactic or therapeutic purposes, or any purchase of Product for resale (alone or as a component) or other commercial purpose, requires a separate license from CST. Customer shall (a) not sell, license, loan, donate or otherwise transfer or make available any Product to any third party, whether alone or in combination with other materials, or use the Products to manufacture any commercial products, (b) not copy, modify, reverse engineer, decompile, disassemble or otherwise attempt to discover the underlying structure or technology of the Products, or use the Products for the purpose of developing any products or services that would compete with CST products or services, (c) not alter or remove from the Products any trademarks, trade names, logos, patent or copyright notices or markings, (d) use the Products solely in accordance with CST Product Terms of Sale and any applicable documentation, and (e) comply with any license, terms of service or similar agreement with respect to any third party products or services used by Customer in connection with the Products.