Revision 6

Store at -20C

#9523

SMAD3 (C67H9) Rabbit mAb

Cell Signaling TECHNOLOGY®

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

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

Product Usage Information

For optimal ChIP and ChIP-seq results, use 10 µl of antibody and 10 µg of chromatin (approximately 4 x 10^6 cells) per IP. This antibody has been validated using SimpleChIP® 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 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #44223.

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-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 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-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).

Background References

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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

W: 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 **Mk:** Monkey

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Limited Uses