Revision 4

Store at -20C

#5678

SMAD2/3 Antibody

Cell Signaling TECHNOLOGY®

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP, IF-IC, FC-FP, ChIP	H M R Mk	Endogenous	52, 60	Rabbit	#P84022, #Q15796	4088, 4087

Product Usage Information

For optimal ChIP 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	1:50
Immunofluorescence (Immunocytochemistry)	1:100
Flow Cytometry (Fixed/Permeabilized)	1:50 - 1:200
Chromatin IP	1:50

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.

Specificity/Sensitivity

SMAD2/3 Antibody recognizes endogenous levels of total SMAD2/3 protein and overexpressed SMAD2/3 protein.

Species predicted to react based on 100% sequence homology

Xenopus

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ala208 of human SMAD2 protein. Antibodies are purified by protein A and peptide affinity chromatography.

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).

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.

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

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat **Mk:** Monkey

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