BACKGROUND

Smads, the only substrates for type I receptor kinases, were first identified as the products of the Drosophila Mad and C. elegans Sma genes. The human genome encodes eight Smad family members (Mad-homologues (MADH)). MADH2, MADH4 and MADH7 map to chromosome 18q21-22, a tumour suppressor locus; MADH3 and MADH6 map to chromosome 15q21-22, and MADH5, MADH1 and MADH8 to chromosomes 15q31, 4 and 13, respectively. Smads are ubiquitously expressed throughout development and in all adult tissues, and many of them (Smad2, Smad4, Smad5, Smad6 and Smad8) are produced from alternatively spliced mRNAs. Functionally, Smads fall into three subfamilies: receptor-activated Smads (R-Smads: Smad1, Smad2, Smad3, Smad5, Smad8), which become phosphorylated by the type I receptors; common mediator Smads (Co-Smads: Smad4), which oligomerise with activated R-Smads; and inhibitory Smads (I-Smads: Smad6 and Smad7), which are induced by TGF-beta family members. The latter exert a negative feedback effect by competing with R-Smads for receptor interaction and by marking the receptors for degradation. Smads have two conserved domains, the N-terminal Mad homology 1 (MH1) and C-terminal Mad homology 2 (MH2) domains. The MH1 domain is highly conserved among R-Smads and Co-Smads; however, the N-terminal parts of I-Smads have only weak sequence similarity to MH1 domains. The MH1 domain regulates nuclear import and transcription by binding to DNA and interacting with nuclear proteins.[1]

Smad proteins transduce signals from transforming growth factor-beta (TGF-beta) superfamily ligands. It has been demonstrated that accessory/scaffolding proteins interact with the type I and II receptors and/or the Smads. One example is SARA (Smad anchor for receptor activation), a cytoplasmic protein that specifically interacts with non-activated Smad2 and the receptor complex, thus forming a bridge between the receptor and Smad2 and assisting in the specific phosphorylation of Smad2 by the type I receptor. The mechanism that organises such Smad signalling centres and its links to receptor endocytosis, degradation and signalling crosstalk could provide cell-context specificity, allowing differential regulation of the basic Smad pathway.[2]

Phosphorylation of the C-terminal serine residues in R-Smads by type I receptor kinases is a crucial step in TGF-beta family signaling. The two most C-terminal serine residues become phosphorylated and, together with a third, non-phosphorylated serine residue, form an evolutionarily conserved SSXS motif in all R-Smads. TGF-beta and activin receptors phosphorylate Smad2 and Smad3, and BMP receptors phosphorylate Smad1, Smad5 and Smad8. Other kinases might also phosphorylate the Smads, which include MAPK, CaMK II and PKC.[3] The phosphorylation at Ser423 and Ser425 of Smad3, which triggers dissociation of Smad3 from its receptors to form a complex with Smad4 and accumulate in the nucleus. Unphosphorylated Smad proteins exist primarily as monomers, and upon phosphorylation, R-Smads form homo-oligomers, which quickly convert to hetero-oligomers containing the Co-Smad, Smad4 and are imported to the nucleus. Nuclear Smad oligomers bind to DNA and associate with transcription factors to regulate expression of target genes. Alternatively, nuclear R-Smads associate with ubiquitin ligases and promote degradation of transcriptional repressors, thus facilitating target gene regulation by TGF-beta. Smads themselves can also become ubiquitinated and are degraded by proteasomes. Finally, the inhibitory Smads (I-Smads) block phosphorylation of R-Smads by the receptors and promote ubiquitination and degradation of receptor complexes, thus inhibiting signaling.[4]

References

1. Feng, X.H. & Derynck, R.: Ann. Rev. Cell Dev. Biol. 21:659-93, 2005
2. Dijke, P.T. & Hill, C.S. : Trends Biochem. Sci. 29:265-73, 2004
3. Massagué, J. et al: Gene Dev. 19:2783-10, 2005
4. Miyazawa, K. et al: Gene. Cell.7:1191-1204, 2002

TECHNICAL INFORMATION

Source:
Smad4 Antibody is a mouse monoclonal antibody raised against purified recombinant human Smad4 fragments expressed in *E. coli*.

Specificity and Sensitivity:
This antibody detects endogenous Smad4 proteins without cross-reactivity with other related proteins.

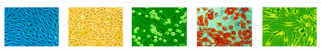
Storage Buffer: PBS and 30% glycerol

Storage:
Store at -20°C for at least one year. Store at 4°C for frequent use. Avoid repeated freeze-thaw cycles.

APPLICATIONS

Application:	*Dilution:
WB	1:1000
IP	1:50
IHC	1:50-200
ICC	1:50-200
FACS	n/d

**Optimal dilutions must be determined by end user.*

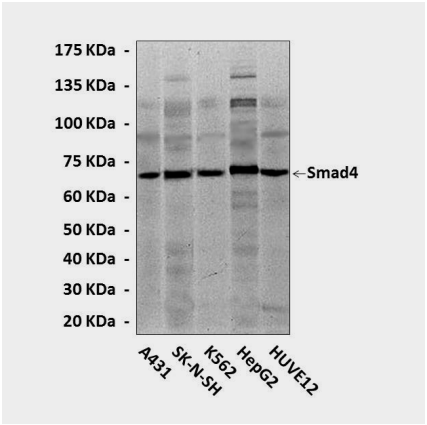


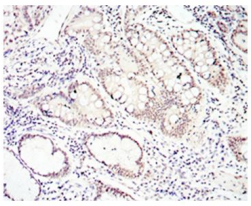
Smad4 Antibody

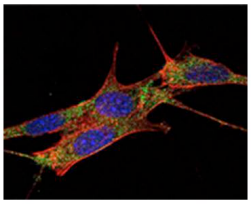
Cat. No. CP10257

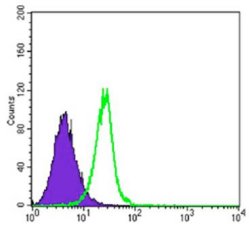
Applications:	**WB, IP, IHC, ICC**
Detected MW:	**70 kDa**
Species & Reactivity:	**Human, Mouse, Rat**
Isotype:	**Mouse IgG1**

QUALITY CONTROL DATA









Top: Western Blot detection of Smad4 proteins in various cell lysates using Smad4 Antibody. **Middle, upper:** This antibody stains paraffin-embedded human lung cancer tissue in immunohistochemical analysis. **Middle, lower:** It also stains NIH3T3 cells in confocal immunofluorescent analysis (Smad4 Antibody: Green; Actin filaments: Red; and DRAQ5: blue). **Bottom:** It also detects Smad4 proteins specifically in K562 cells by FACS assay (Smad4 Antibody: Green and negative control IgG: Blue).