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**SMAD4 (D3R4N) XP® Rabbit mAb****Cell Signaling**  
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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:
WB, IP, IHC-Bond, IHC-P, IF-IC, FC-FP, ChIP, ChIP-seq	H M R Mk	Endogenous	70	Rabbit IgG	#Q13485	4089

**Product Usage Information**

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

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:200
IHC Leica Bond	1:400 - 1:1600
Immunohistochemistry (Paraffin)	1:200 - 1:800
Immunofluorescence (Immunocytochemistry)	1:400 - 1:1600
Flow Cytometry (Fixed/Permeabilized)	1:400 - 1:1600
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 #73885.

**Specificity / Sensitivity**

SMAD4 (D3R4N) XP® Rabbit mAb recognizes endogenous levels of total SMAD4 protein.

**Source / Purification**

Monoclonal antibody is produced by immunizing animals with recombinant protein corresponding to human SMAD4 protein. The epitope has been mapped to residues surrounding Ser315.

**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, 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 SSXS motif in the carboxy-terminus. 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 nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

**Applications Key**

**WB:** Western Blotting **IP:** Immunoprecipitation **IHC-Bond:** IHC Leica Bond  
**IHC-P:** Immunohistochemistry (Paraffin) **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

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