Revision 3

#12534 Store at -20C

# SMAD5 (D4G2) Rabbit mAb

Cell Signaling TECHNOLOGY®

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| Applications: | Reactivity: | Sensitivity: | MW (kDa): | Source/Isotype: | UniProt ID: | Entrez-Gene Id: |
|---|---|---|---|---|---|---|
| WB, IP, ChIP | H M R Mk | Endogenous | 60 | Rabbit IgG | #Q99717 | 4090 |

## Product Usage Information

For optimal ChIP results, use 5 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 |
| Chromatin IP | 1:100 |

## 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.

## Specificity / Sensitivity

SMAD5 (D4G2) Rabbit mAb recognizes endogenous levels of total SMAD5 protein.

## Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro249 of human SMAD5 protein.

## Background

Bone morphogenetic proteins (BMPs) constitute a large family of signaling molecules that regulate a wide range of critical processes including morphogenesis, cell-fate determination, proliferation, differentiation, and apoptosis (1,2). BMP receptors are members of the TGF-β superfamily of Ser/Thr kinase receptors. Ligand binding induces multimerization, autophosphorylation, and activation of these receptors (3-5). They subsequently phosphorylate SMAD1 at Ser463 and Ser465 in the carboxy-terminal motif SSXS, as well as SMAD5 and SMAD9 (SMAD8) at their corresponding sites. These phosphorylated SMADs dimerize with the coactivating SMAD4 and translocate to the nucleus, where they regulate the transcription of target genes (5). MAP kinases and CDKs 8 and 9 are also reported to phosphorylate residues in the linker region of SMAD1, including Ser206. Phosphorylation of SMAD1 at Ser206 recruits Smurf1 to the linker region and leads to the degradation of SMAD1 (6). Phosphorylation at this site also promotes SMAD1 transcriptional activity by recruiting YAP to the linker region (7).

## Background References

1. Hogan, B.L. (1996) *Genes Dev* 10, 1580-94.
2. Hoodless, P.A. et al. (1996) *Cell* 85, 489-500.
3. Klemm, J.D. et al. (1998) *Annu Rev Immunol* 16, 569-92.
4. Kretzschmar, M. et al. (1997) *Genes Dev* 11, 984-95.
5. Whitman, M. (1998) *Genes Dev* 12, 2445-62.
6. Sapkota, G. et al. (2007) *Mol Cell* 25, 441-54.
7. Alarcón, C. et al. (2009) *Cell* 139, 757-69.

## 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 **ChIP:** Chromatin IP

## 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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