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[KO Validated] SMAD2/SMAD3 Rabbit mAb

Catalog No.: A28421 KO Validated Recombinant

Basic Information

Observed MW

52 kDa/60 kDa

Calculated MW

25-52 kDa

Category

Primary antibody

Applications

WB,IP,ChIP,ELISA

Cross-Reactivity

Human, Mouse, Rat

CloneNo number

ARC77767

ELISA

Recommended Dilutions

WB 1:6000 - 1:15000

ΙP 0.5μg-4μg antibody for 200µg-400µg extracts of

whole cells

2µg antibody for ChIP 10μg-15μg of Chromatin

Recommended starting

concentration is 1 µg/mL. Please optimize the concentration based on your specific assay requirements.For highratio antibody dilutions (≥1:10000)∏a sequential dilution method is

strongly recommended to ensure measurement accuracy.

Background

The protein encoded by this gene belongs to the SMAD, a family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (Mad) and the C. elegans gene Sma. SMAD proteins are signal transducers and transcriptional modulators that mediate multiple signaling pathways. This protein mediates the signal of the transforming growth factor (TGF)-beta, and thus regulates multiple cellular processes, such as cell proliferation, apoptosis, and differentiation. This protein is recruited to the TGF-beta receptors through its interaction with the SMAD anchor for receptor activation (SARA) protein. In response to TGF-beta signal, this protein is phosphorylated by the TGF-beta receptors. The phosphorylation induces the dissociation of this protein with SARA and the association with the family member SMAD4. The association with SMAD4 is important for the translocation of this protein into the nucleus, where it binds to target promoters and forms a transcription repressor complex with other cofactors. This protein can also be phosphorylated by activin type 1 receptor kinase, and mediates the signal from the activin. Alternatively spliced transcript variants have been observed for this gene. The SMAD family of proteins are a group of intracellular signal transducer proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (Mad) and the C. elegans gene Sma. The SMAD3 protein functions in the transforming growth factor-beta signaling pathway, and transmits signals from the cell surface to the nucleus, regulating gene activity and cell proliferation. This protein forms a complex with other SMAD proteins and binds DNA, functioning both as a transcription factor and tumor suppressor. Mutations in this gene are associated with aneurysms-osteoarthritis syndrome and Loeys-Dietz Syndrome 3.

Immunogen Information

Gene ID Swiss Prot 4087/4088 Q15796/P84022

Immunogen

Synthetic peptide. This information is considered to be commercially sensitive.

Synonyms

CHTD8; JV18; JV18-1; LDS6; MADH2; MADR2; hMAD-2; hSMAD2; HSPC193; HsT17436; JV15-2; LDS1C; LDS3; MADH3; hMAD-3; hSMAD3; mad3

Product Information

Source Isotype **Purification** Rabbit Affinity purification IgG

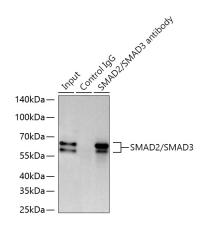
Storage

Store at -20°C. Avoid freeze / thaw cycles.

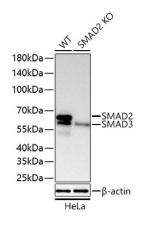
Buffer: PBS with 0.09% Sodium azide, 0.05% BSA, 50% glycerol, pH7.3.

Contact

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Immunoprecipitation of SMAD2/SMAD3 from 300 μg extracts of NIH/3T3 cells was performed using 0.5 μg of [KO Validated] SMAD2/SMAD3 Rabbit mAb (A28421). Rabbit Control IgG (AC005) was used to precipitate the Control IgG sample. IP samples were eluted with 1x Laemmli Buffer. The Input lane represents 10% of the total input. Western blot analysis of immunoprecipitates was conducted using [KO Validated] SMAD2/SMAD3 Rabbit mAb (A28421) at a dilution of 1:6000.

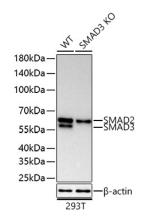


Western blot analysis of lysates from wild type (WT) and SMAD2/SMAD3 knockout (KO) HeLa cells using [KO Validated] SMAD2/SMAD3 Rabbit mAb (A28421) at 1:15000 dilution incubated overnight at 4° C. Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution. Lysates/proteins: 25 µg per lane.

Blocking buffer: 3% nonfat dry milk in TBST.

Detection: ECL Basic Kit (RM00020).

Exposure time: 45 s.

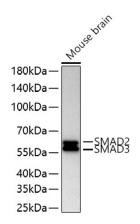


Western blot analysis of lysates from wild type (WT) and SMAD2/SMAD3 knockout (KO) 293T cells using [KO Validated] SMAD2/SMAD3 Rabbit mAb (A28421) at 1:15000 dilution incubated overnight at 4° C. Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution.

Lysates/proteins: 25 µg per lane. Blocking buffer: 3% nonfat dry milk in TBST.

Blocking buffer: 3% nonfat dry milk in TBS Detection: ECL Basic Kit (RM00020).

Exposure time: 45 s.



Western blot analysis of lysates from Mouse brain using [KO Validated] SMAD2/SMAD3 Rabbit mAb (A28421) at 1:15000 dilution incubated overnight at 4°C.

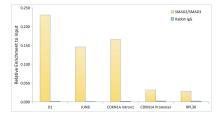
Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution.

Lysates/proteins: 25 µg per lane.

Blocking buffer: 3% nonfat dry milk in TBST.

Detection: ECL Basic Kit (RM00020).

Exposure time: 45 s.



Chromatin immunoprecipitation was performed with 15 μ g of cross-linked chromatin from A549 cells treated with TGF- β 1 (20 ng/mL,30 min) , using 2 μ g of [KO Validated] SMAD2/SMAD3 Rabbit mAb (A28421) and Rabbit IgG isotype control (AC042). The enrichment of immunoprecipitated DNA at different genomic loci was examined by quantitative PCR. The histogram compares the ratio of the immunoprecipitated DNA to the input at given loci.