Anti-Smad2 + Smad3 Antibody [PSH21-36]

HA724174

Product Type:	Recombinant Rabbit multiclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 52/48 kDa
Clone number:	PSH21-36

Description: Mothers against decapentaplegic homolog 2, also known as SMAD family member 2 or SMAD2, is a protein that in humans is encoded by the SMAD2 gene. MAD homolog 2 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. Mothers against decapentaplegic homolog 3 also known as SMAD family member 3 or SMAD3 is a protein that in humans is encoded by the SMAD3 gene. SMAD3 is a member of the SMAD family of proteins. It acts as a mediator of the signals initiated by the transforming growth factor beta (TGF-β) superfamily of cytokines, which regulate cell proliferation, differentiation and death. Based on its essential role in TGF beta signaling pathway, SMAD3 has been related with tumor growth in cancer development.

Positive control: HeLa cell lysate, C6 cell lysate, Mouse lung tissue lysate, NIH/3T3, C6.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: Q15796 Human | P84022 Human | Q62432 Mouse | Q8BUN5 Mouse | O70436 Rat | P84025 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:100
FC	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

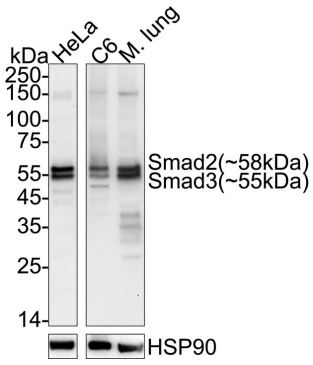

Fig1: Western blot analysis of Smad2 + Smad3 on different lysates with Rabbit anti-Smad2 + Smad3 antibody (HA724174) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: C6 cell lysate (20 µg/Lane)
Lane 3: Mouse lung tissue lysate (40 µg/Lane)

Predicted band size: 52/48 kDa
Observed band size: 58/55 kDa

Exposure time: 59 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA724174) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

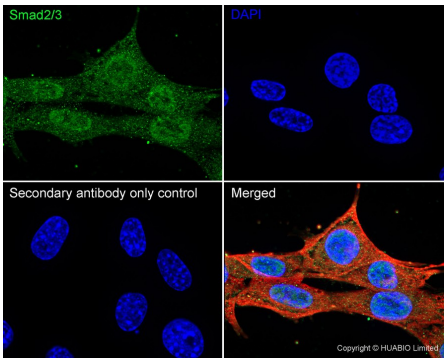

Fig2: Immunocytochemistry analysis of NIH/3T3 cells labeling Smad2 + Smad3 with Rabbit anti-Smad2 + Smad3 antibody (HA724174) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Smad2 + Smad3 antibody (HA724174) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

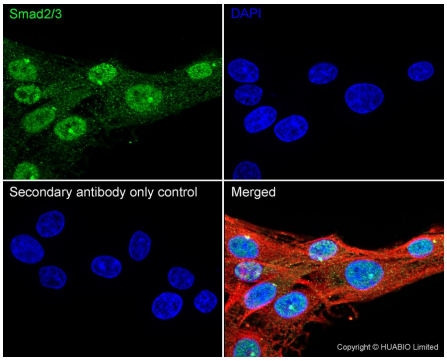

Fig3: Immunocytochemistry analysis of C6 cells labeling Smad2 + Smad3 with Rabbit anti-Smad2 + Smad3 antibody (HA724174) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Smad2 + Smad3 antibody (HA724174) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

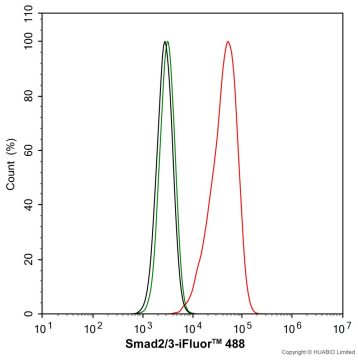

Fig4: Flow cytometric analysis of NIH/3T3 cells labeling Smad2 + Smad3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA724174, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Zhang L et al. Creatine promotes cancer metastasis through activation of Smad2/3. Cell Metab. 2021 Jun
2. Feng Y et al. BRD9-SMAD2/3 Orchestrates Stemness and Tumorigenesis in Pancreatic Ductal Adenocarcinoma. Gastroenterology. 2024 Jan