Anti-Phospho-SMAD2 (S465+467) Antibody [PSH05-76] HA722443

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 52/48 kDa
Clone number:	PSH05-76

Description: R-SMADs are receptor-regulated SMADs. SMADs are transcription factors that transduce extracellular TGF-β superfamily ligand signaling from cell membrane bound TGF-β receptors into the nucleus where they activate transcription TGF-β target genes. R-SMADS are directly phosphorylated on their c-terminus by type 1 TGF-β receptors through their intracellular kinase domain, leading to R-SMAD activation. R-SMADS include SMAD2 and SMAD3 from the TGF-β/Activin/Nodal branch, and SMAD1, SMAD5 and SMAD8 from the BMP/GDP branch of TGF-β signaling. In response to signals by the TGF-β superfamily of ligands these proteins associate with receptor kinases and are phosphorylated at an SSXS motif at their extreme C-terminus. These proteins then typically bind to the common mediator Smad or co-SMAD SMAD4.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser465 and 467 of Human SMAD2.

Positive control: HeLa cell lysate, HeLa starved overnight then treated with 10ng/mL TGF-β1 for 30 minutes cell lysate, NIH/3T3 starved overnight then treated with 10ng/mL TGF-β1 for 30 minutes cell lysate, HeLa cells treated with 20ng/mL TGF-β1 for 15 minutes, HeLa, NIH/3T3, human stomach tissue, rat lung tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: Q15796 Human | Q62432 Mouse | O70436 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:500
IHC-P	1:200

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

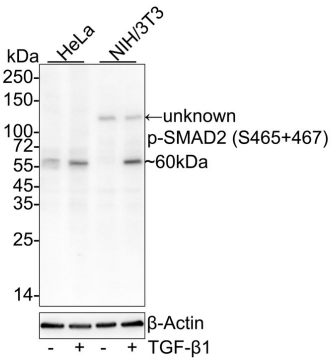

Fig1: Western blot analysis of Phospho-SMAD2 (S465+467) on different lysates with Rabbit anti-Phospho-SMAD2 (S465+467) antibody (HA722443) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa starved overnight then treated with 10ng/mL TGF-β1 for 30 minutes cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: NIH/3T3 starved overnight then treated with 10ng/mL TGF-β1 for 30 minutes cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 52/48 kDa
Observed band size: 60 kDa

Exposure time: 1 minute 20 seconds; ECL: K1802;

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 (HA722443) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

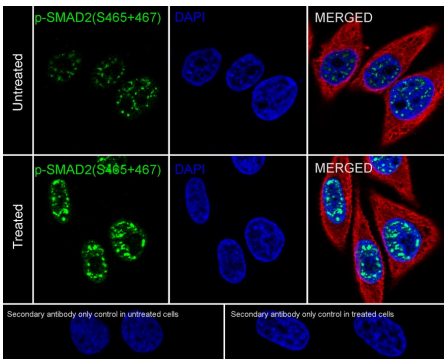

Fig2: Immunocytochemistry analysis of HeLa cells treated with or without 20ng/mL TGF-β1 for 15 minutes labeling Phospho-SMAD2 (S465+467) with Rabbit anti-Phospho-SMAD2 (S465+467) antibody (HA722443) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Phospho-SMAD2 (S465+467) antibody (HA722443) at 1/500 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 (M1305-2, 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.

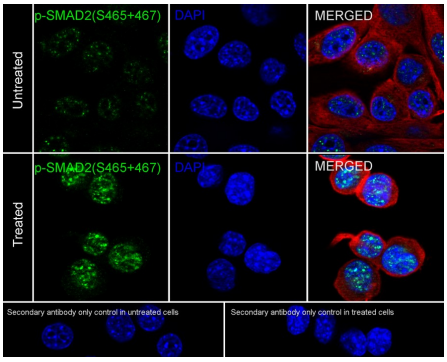

Fig3: Immunocytochemistry analysis of NIH/3T3 cells treated with or without 20ng/mL TGF-β1 for 15 minutes labeling Phospho-SMAD2 (S465+467) with Rabbit anti-Phospho-SMAD2 (S465+467) antibody (HA722443) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Phospho-SMAD2 (S465+467) antibody (HA722443) at 1/500 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.

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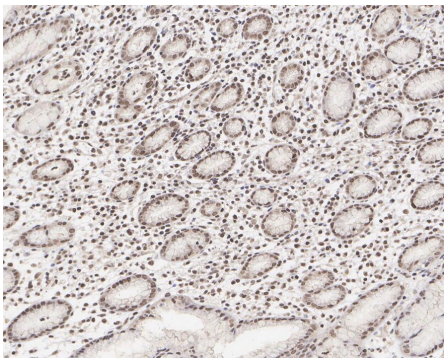

Fig4: Immunohistochemical analysis of paraffin-embedded human stomach tissue with Rabbit anti-Phospho-SMAD2 (S465+467) antibody (HA722443) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722443) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

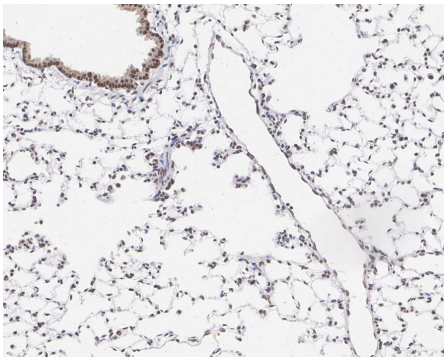

Fig5: Immunohistochemical analysis of paraffin-embedded rat lung tissue with Rabbit anti-Phospho-SMAD2 (S465+467) antibody (HA722443) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722443) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Yin H et al. M6A RNA methylation-mediated RMRP stability renders proliferation and progression of non-small cell lung cancer through regulating TGFBR1/SMAD2/SMAD3 pathway. Cell Death Differ. 2023 Mar
2. Yang S et al. SIRT2 alleviated renal fibrosis by deacetylating SMAD2 and SMAD3 in renal tubular epithelial cells. Cell Death Dis. 2023 Sep