Anti-Phospho-Smad1/5/9 (S463/S465/S467) Antibody [JE59-46]

HA722566

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 52 kDa
Clone number:	JE59-46

Description: Smads (or SMADs) comprise a family of structurally similar proteins that are the main signal transducers for receptors of the transforming growth factor beta (TGF-B) superfamily, which are critically important for regulating cell development and growth. There are three distinct sub-types of Smads: receptor-regulated Smads (R-Smads), common partner Smads (Co-Smads), and inhibitory Smads (I-Smads). The eight members of the Smad family are divided among these three groups. Trimers of two receptor-regulated SMADs and one co-SMAD act as transcription factors that regulate the expression of certain genes. The R-Smads consist of Smad1, Smad2, Smad3, Smad5 and Smad8/9, and are involved in direct signaling from the TGF-B receptor. R/Co-Smads are primarily located in the cytoplasm, but accumulate in the nucleus following TGF-β signaling, where they can bind to DNA and regulate transcription. However, I-Smads are predominantly found in the nucleus, where they can act as direct transcriptional regulators.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Ser463/465 of human SMAD1 and SMAD5 protein.

Positive control: HeLa treated with 10ng/mL BMP4 for 1 hour cell lysate, NIH/3T3 treated with 10ng/mL BMP4 for 1 hour cell lysate, human testis tissue, C6 treated with 10ng/mL BMP4 for 1 hour cell lysate, HeLa cells treated with 10ng/mL BMP4 for 1 hour, NIH/3T3 cells treated with 10ng/mL BMP4 for 1 hour.

Subcellular location: Cytoplasm, Nucleus, Mitochondrion.

Database links: SwissProt: Q15797 Human | Q99717 Human | O15198 Human | P70340 Mouse | P97454 Mouse | Q9JIW5 Mouse | O54835 Rat | P97588 Rat | Q9R1V3 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:1,000
IF-Cell	1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% 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

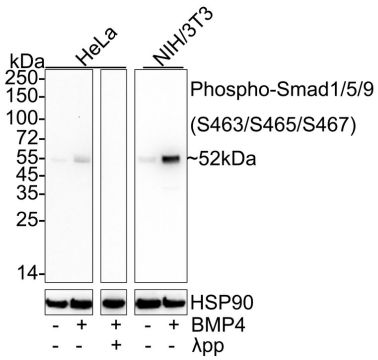

Fig1: Western blot analysis of Phospho-Smad1/5/9 (S463/S465/S467) on different lysates with Rabbit anti-Phospho-Smad1/5/9 (S463/S465/S467) antibody (HA722566) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 10ng/mL BMP4 for 1 hour cell lysate
Lane 3: HeLa treated with 10ng/mL BMP4 for 1 hour cell lysate, then the membrane treated with λpp for 1 hour
Lane 4: NIH/3T3 cell lysate
Lane 5: NIH/3T3 treated with 10ng/mL BMP4 for 1 hour cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 52 kDa
Observed band size: 52 kDa

Exposure time: Lane 1-3: 3 minutes; Lane 4-5: 42 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 (HA722566) 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.

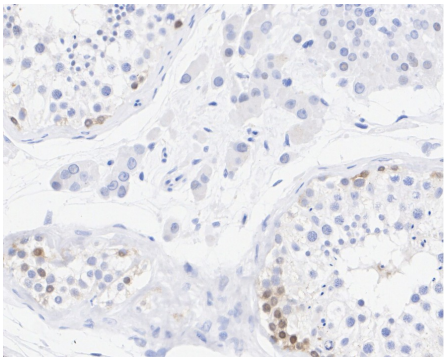

Fig2: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-Phospho-Smad1/5/9 (S463/S465/S467) antibody (HA722566) at 1/1,000 dilution.

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 (HA722566) at 1/1,000 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.

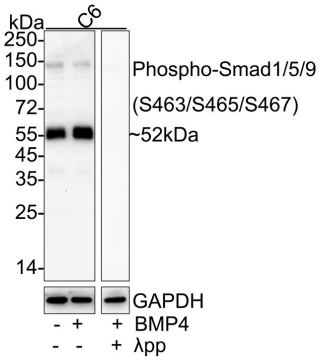

Fig3: Western blot analysis of Phospho-Smad1/5/9 (S463/S465/S467) on different lysates with Rabbit anti-Phospho-Smad1/5/9 (S463/S465/S467) antibody (HA722566) at 1/1,000 dilution.

Lane 1: C6 cell lysate
Lane 2: C6 treated with 10ng/mL BMP4 for 1 hour cell lysate
Lane 3: C6 treated with 10ng/mL BMP4 for 1 hour cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 10 µg/Lane.

Predicted band size: 52 kDa
Observed band size: 52 kDa

Exposure time: 1 minute 18 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 (HA722566) 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.

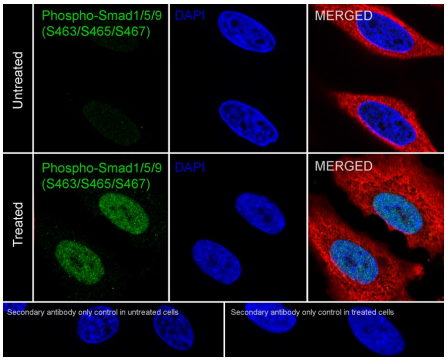

Fig4: Immunocytochemistry analysis of HeLa cells treated with 10ng/mL BMP4 for 1 hour labeling Phospho-Smad1/5/9 (S463/S465/S467) with Rabbit anti-Phospho-Smad1/5/9 (S463/S465/S467) antibody (HA722566) at 1/100 dilution.

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-Smad1/5/9 (S463/S465/S467) antibody (HA722566) 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 (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.

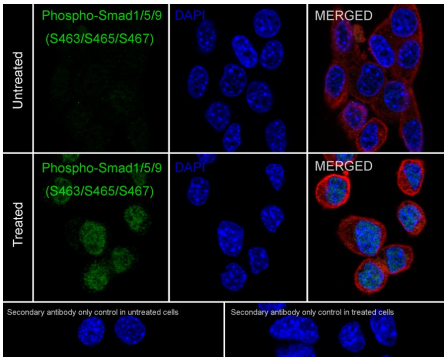

Fig5: Immunocytochemistry analysis of NIH/3T3 cells treated with 10ng/mL BMP4 for 1 hour labeling Phospho-Smad1/5/9 (S463/S465/S467) with Rabbit anti-Phospho-Smad1/5/9 (S463/S465/S467) antibody (HA722566) at 1/100 dilution.

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-Smad1/5/9 (S463/S465/S467) antibody (HA722566) 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 (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.

Background References

1. Nakatsu D et al. BMP4-SMAD1/5/9-RUNX2 pathway activation inhibits neurogenesis and oligodendrogenesis in Alzheimer's patients' iPSCs in senescence-related conditions. Stem Cell Reports. 2023 Mar
2. Nakajima T et al. Changes in Smad1/5/9 expression and phosphorylation in astrocytes of the rat hippocampus after transient global cerebral ischemia. J Chem Neuroanat. 2021 Apr