Anti-Smad3 Antibody [SY25-01]

ET1607-41

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 48 kDa
Clone number:	SY25-01

Description: Smad proteins, the mammalian homologs of the Drosophila Mothers against dpp (Mad) have been implicated as downstream effectors of TGFβ/BMP signaling. Smad1 (also designated Madr1 or JV4-1), Smad5 and mammalian Smad8 (also designated Smad9 or MADH6) are effectors of BMP2 and BMP4 function while Smad2 (also designated Madr2 or JV18-1) and Smad3 are involved in TGFβ and activin-mediated growth modulation. Smad4 (also designated DPC4) has been shown to mediate all of the above activities through interaction with various Smad family members. Smad6 and Smad7 regulate the response to activin/TGFβ signaling by interfering with TGFβ-mediated phosphorylation of other Smad family members.

Immunogen: Synthetic peptide within Human Smad3 aa 181-230 / 425.

Positive control: HeLa cell lysate, A549 cell lysate, HL-60 cell lysate, NIH/3T3 cell lysate, bEnd.3 cell lysate, C6 cell lysate, Wild-type HaCaT whole cell lysate, A549, human lung carcinoma tissue, human breast carcinoma tissue, mouse liver tissue, mouse brain tissue, rat testis tissue, HeLa.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P84022 Human | Q8BUN5 Mouse | P84025 Rat

Recommended Dilutions:

WB	1:5,000-1:20,000
IF-Cell	1:500
IF-Tissue	1:50-1:200
IHC-P	1:50-1:1,000
FC	1:1,000

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

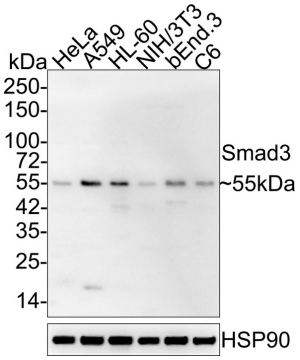

Fig1: Western blot analysis of Smad3 on different lysates with Rabbit anti-Smad3 antibody (ET1607-41) at 1/20,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: A549 cell lysate
Lane 3: HL-60 cell lysate
Lane 4: NIH/3T3 cell lysate
Lane 5: bEnd.3 cell lysate
Lane 6: C6 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 48 kDa
Observed band size: 55 kDa

Exposure time: 1 minute 52 seconds;

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 (ET1607-41) at 1/20,000 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.

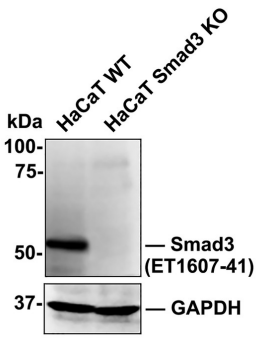

Fig2: All lanes: Western blot analysis of Smad3 with anti-Smad3 antibody (ET1607-41) at 1:1,000 dilution.
Lane 1: Wild-type HaCaT whole cell lysate (15 µg).
Lane 2: Smad3 knockout HaCaT whole cell lysate (15 µg).

ET1607-41 was shown to specifically react with Smad3 in wild-type HaCaT cells. NO band was observed when Smad3 knockout sample was tested. Wild-type and Smad3 knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1607-41, 1:1,000) was used in 5% BSA at room temperature for 2 hours. Goat anti-Rabbit IgG-HRP antibody at 1:10,000 dilution was used for 1 hour at room temperature.

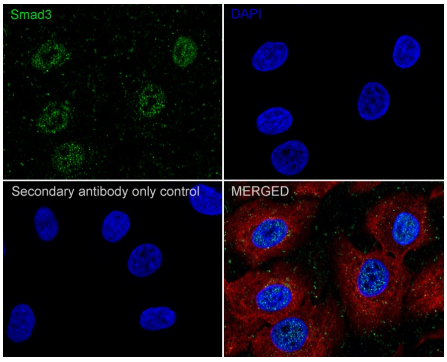

Fig3: Immunocytochemistry analysis of A549 cells labeling Smad3 with Rabbit anti-Smad3 antibody (ET1607-41) at 1/500 dilution.

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-Smad3 antibody (ET1607-41) 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.

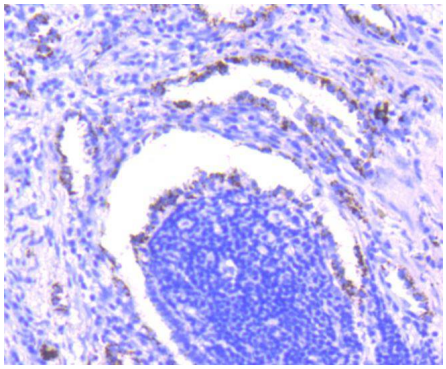

Fig4: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue using anti-Smad3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-41, 1/50) for 30 minutes 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.

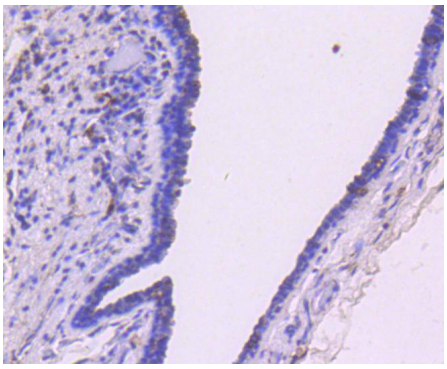

Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Smad3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-41, 1/50) for 30 minutes 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.

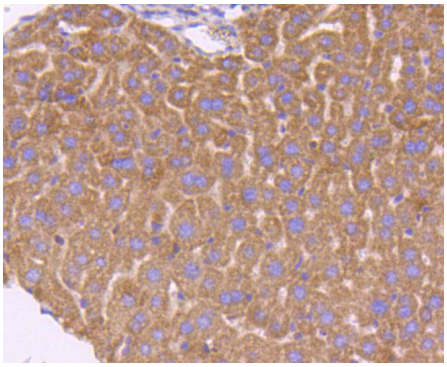

Fig6: Immunohistochemical analysis of paraffin-embedded mouse liver tissue using anti-Smad3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-41, 1/50) for 30 minutes 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.

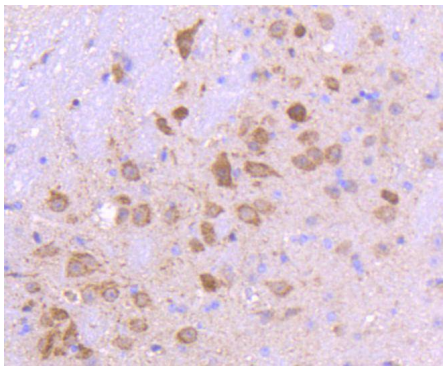

Fig7: Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-Smad3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-41, 1/50) for 30 minutes 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.

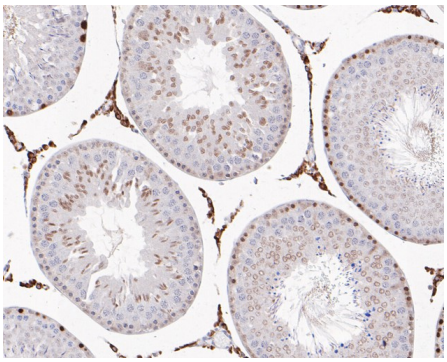

Fig8: Immunohistochemical analysis of paraffin-embedded rat testis tissue using anti-Smad3 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-41, 1/200) for 30 minutes 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.

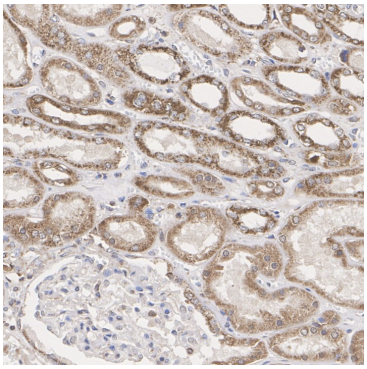

Fig9: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Smad3 antibody (ET1607-41) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 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 (ET1607-41) 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.

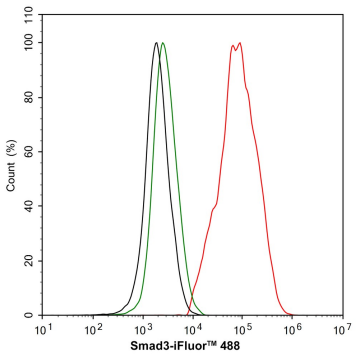

Fig10: Flow cytometric analysis of HeLa cells labeling Smad3.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1607-41, 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. Dai X et al. SMAD3 deficiency promotes vessel wall remodeling, collagen fiber reorganization and leukocyte infiltration in an inflammatory abdominal aortic aneurysm mouse model. Sci Rep 5:10180 (2015).
2. Chen JL et al. Development of novel activin-targeted therapeutics. Mol Ther 23:434-44 (2015).