Anti-Smad4 Antibody [SP05-05]

ET1604-12

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: 60 kDa
Clone number:	SP05-05

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 C-terminal human Smad4.

Positive control: HeLa cell lysate, HCT 116 cell lysate, C6 cell lysate, Neuro-2a cell lysates, HeLa, Neuro-2a, PC-12, human colon tissue, human lung tissue, mouse lung tissue, rat lung tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: Q13485 Human | P97471 Mouse | O70437 Rat

Recommended Dilutions:

WB	1:1,000-1:5,000
IF-Cell	1:100
IF-Tissue	1:50-1:200
IHC-P	1:200
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

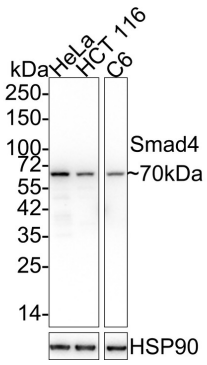


Fig1: Western blot analysis of Smad4 on different lysates with Rabbit anti-Smad4 antibody (ET1604-12) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HCT 116 cell lysate
Lane 3: C6 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 60 kDa
Observed band size: 70 kDa

Exposure time: 3 minutes;

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 (ET1604-12) at 1/5,000 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.

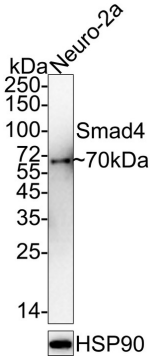


Fig2: Western blot analysis of Smad4 on Neuro-2a cell lysates with Rabbit anti-Smad4 antibody (ET1604-12) at 1/1,000 dilution.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 60 kDa
Observed band size: 70 kDa

Exposure time: 3 minutes; 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 (ET1604-12) 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.

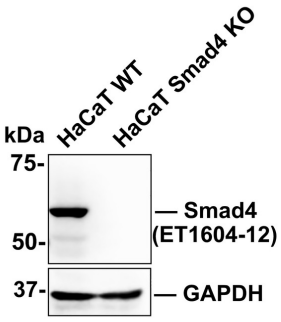
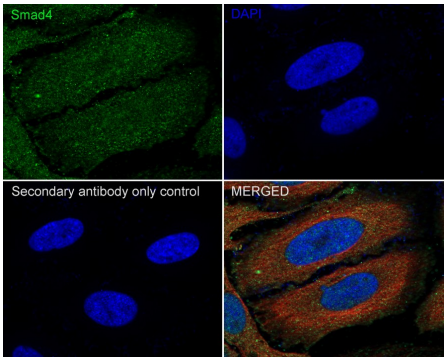


Fig3: All lanes: Western blot analysis of Smad4 with anti-Smad4 antibody (ET1604-12) at 1:1,000 dilution.
Lane 1: Wild-type HaCaT whole cell lysate (15 µg).
Lane 2: Smad4 knockout HaCaT whole cell lysate (15 µg).

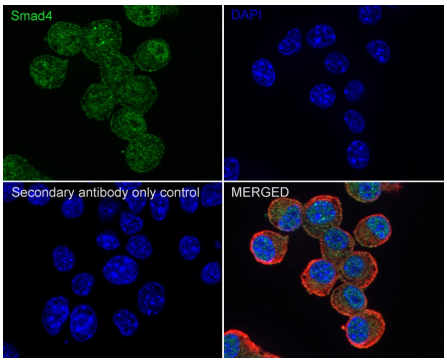
ET1604-12 was shown to specifically react with Smad4 in wild-type HaCaT cells. NO band was observed when Smad4 knockout sample was tested. Wild-type and Smad4 knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1604-12, 1:1,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody at 1:10,000 dilution was used for 1 hour at room temperature.

Fig4: Immunocytochemistry analysis of HeLa cells labeling Smad4 with Rabbit anti-Smad4 antibody (ET1604-12) 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-Smad4 antibody (ET1604-12) 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 Neuro-2a cells labeling Smad4 with Rabbit anti-Smad4 antibody (ET1604-12) 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-Smad4 antibody (ET1604-12) 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.

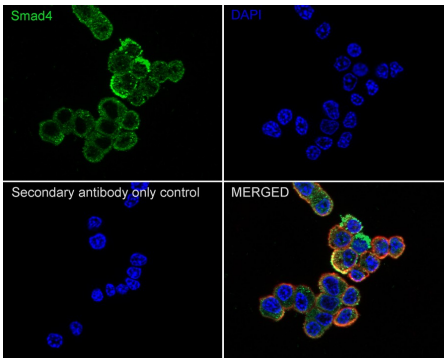


Fig6: Immunocytochemistry analysis of PC-12 cells labeling Smad4 with Rabbit anti-Smad4 antibody (ET1604-12) at 1/100 dilution.

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-Smad4 antibody (ET1604-12) 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.

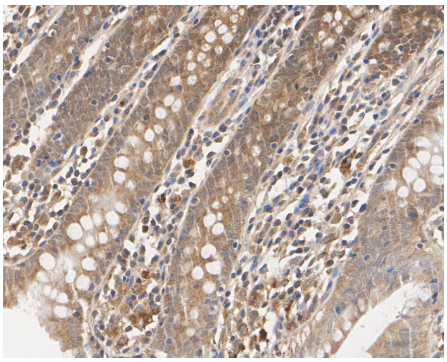


Fig7: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-Smad4 antibody (ET1604-12) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1604-12) 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.

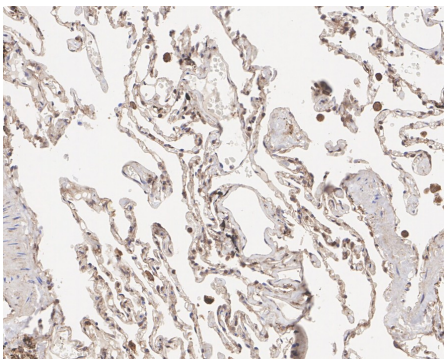


Fig8: Immunohistochemical analysis of paraffin-embedded human lung tissue with Rabbit anti-Smad4 antibody (ET1604-12) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1604-12) 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.

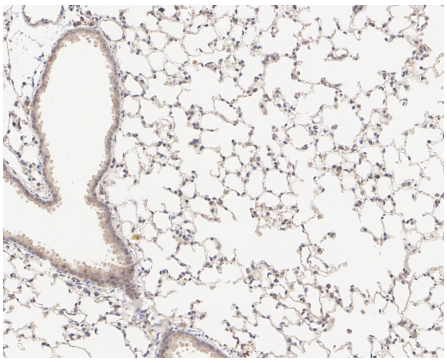


Fig9: Immunohistochemical analysis of paraffin-embedded mouse lung tissue with Rabbit anti-Smad4 antibody (ET1604-12) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1604-12) 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.

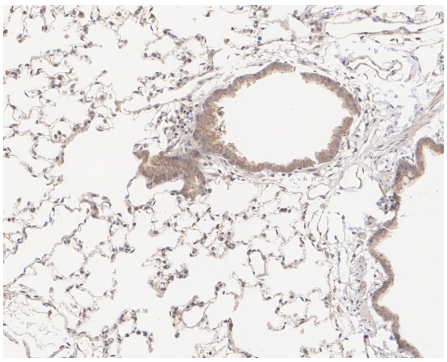


Fig10: Immunohistochemical analysis of paraffin-embedded rat lung tissue with Rabbit anti-Smad4 antibody (ET1604-12) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1604-12) 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.

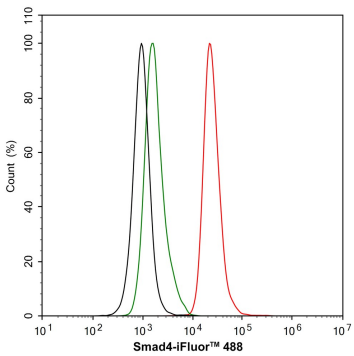


Fig11: Flow cytometric analysis of HeLa cells labeling Smad4.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1604-12, 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).

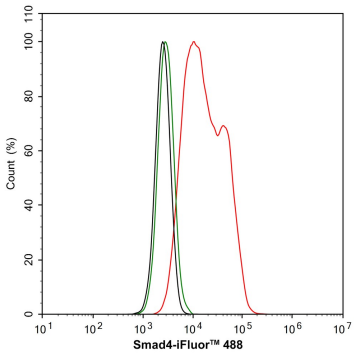


Fig12: Flow cytometric analysis of Neuro-2a cells labeling Smad4.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1604-12, 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).

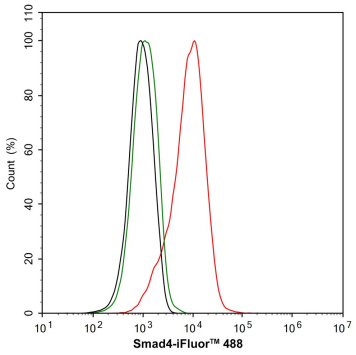


Fig13: Flow cytometric analysis of PC-12 cells labeling Smad4.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1604-12, 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. Maity, G. et al. 2015. Aspirin blocks growth of breast tumor cells and tumor-initiating cells and induces reprogramming factors of mesenchymal to epithelial transition. Laboratory investigation; a journal of technical methods and pathology. 95: 702-17.
2. Voorneveld, PW. et al. 2015. The BMP pathway either enhances or inhibits the Wnt pathway depending on the SMAD4 and p53 status in CRC. Br. J. Cancer. 112: 122-30.