Anti-Smad4 Antibody [PD01-37]

HA721234

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 60 kDa
Clone number:	PD01-37

Description: The SMAD4 gene is located on chromosome 18q21 and encodes the SMAD4 protein belonging to the SMAD family of transcription factor proteins, which act as mediators of TGF-β signal transduction. The TGF-β/SMAD4 signaling pathway controls signal transduction from cell membrane to nucleus, and is responsible for a wide range of cellular processes such as proliferation, differentiation, apoptosis, migration, as well as cancer initiation and progression. Alterations in the SMAD4 gene was primarily discovered in pancreatic cancer (duct adenocarcinoma) but occur in a variety of cancers such as colorectal cancer, gastric cancer, prostate cancer, melanomas, head and neck cancers and many others, though with higher frequencies in gastrointestinal tract cancers. Loss of SMAD4 expression in tumors has also been shown to affect cancer progression and therapy, such as reduced response to adjuvant chemotherapy. Tonsil is recommendable as external control for SMAD4: The vast majority of basal squamous epithelial cells in the surface epithelium must show a strong, predominantly nuclear staining reaction, whereas superficial squamous epithelial cells typically should be negative or only faintly demonstrated. Virtually all other cells e.g. lymphocytes must show an at least moderate, predominantly nuclear but also cytoplasmic staining reaction. In addition, a tumor (pancreas or colon) with loss of SMAD4 due to inactivation of the gene could be included, in which stromal cells (internal tissue control) should display the required staining reaction - at least moderate intensity. However, for SMAD4 it has to be emphasized that internal positive tissue controls being e.g. normal stromal cells adjacent to the neoplastic cells are preferred to external controls. An observed intact nuclear expression of SMAD4 proteins in the internal normal cells together with loss of SMAD4 proteins in the neoplastic cells is diagnostic essential.

Immunogen:	Synthetic peptide within Human Smad4 aa 500 to the C-terminus (C terminal).
Positive control:	HepG2 cell lysate, Jurkat cell lysate, SH-SY5Y cell lysate, Ramos cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, C6 cell lysate, PANC-1, human lung carcinoma tissue, human testis tissue, human stomach carcinoma tissue.
Subcellular location:	Cytoplasm, Nucleus.
Database links:	SwissProt: Q13485 Human | P97471 Mouse | O70437 Rat
Recommended Dilutions:	
WB	1:1,000
IHC-P	1:1,000
IF-Cell	1:50
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

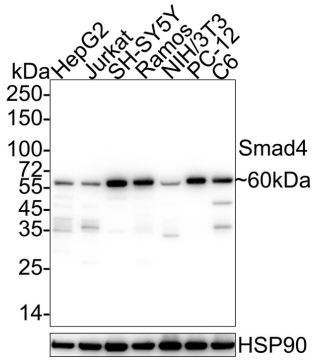

Fig1: Western blot analysis of Smad4 on different lysates with Rabbit anti-Smad4 antibody (HA721234) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate
Lane 2: Jurkat cell lysate
Lane 3: SH-SY5Y cell lysate
Lane 4: Ramos cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: PC-12 cell lysate
Lane 7: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 25 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 (HA721234) 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.

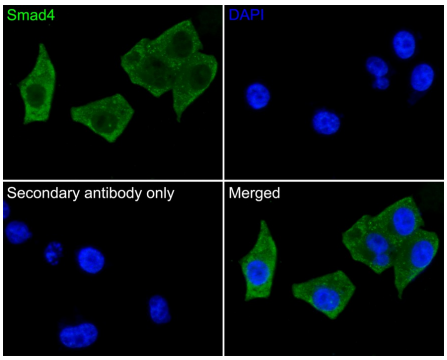

Fig2: Immunocytochemistry analysis of PANC-1 cells labeling Smad4 with Rabbit anti-Smad4 antibody (HA721234) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Smad4 antibody (HA721234) at 1/50 dilution in 2% negative goat serum 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.

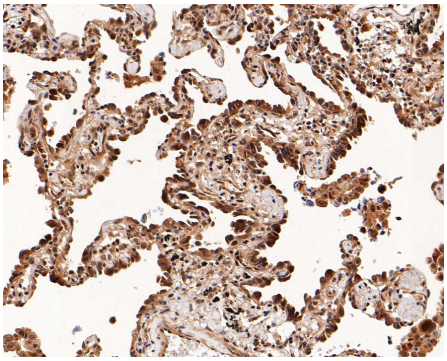

Fig3: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue with Rabbit anti-Smad4 antibody (HA721234) at 1/1,000 dilution.

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 (HA721234) 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.

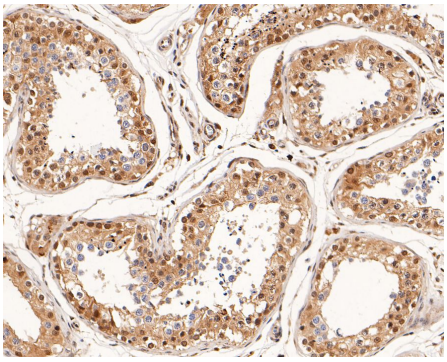

Fig4: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-Smad4 antibody (HA721234) at 1/1,000 dilution.

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 (HA721234) 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.

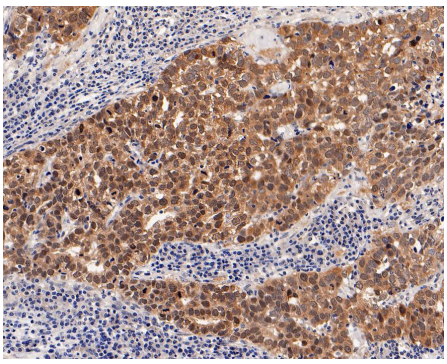

Fig5: Immunohistochemical analysis of paraffin-embedded human stomach carcinoma tissue with Rabbit anti-Smad4 antibody (HA721234) at 1/1,000 dilution.

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 (HA721234) 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.

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.