abcam

Product datasheet

Anti-Smad4 antibody [EP618Y] ab40759

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Smad4 antibody [EP618Y]
Description	Rabbit monoclonal [EP618Y] to Smad4
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P **Unsuitable for:** Flow Cyt,ICC/IF or IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Smad4 aa 500 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: **Q13485** (Peptide available as **ab228416**)
Positive control	WB: Wild type HAP1 whole cell lysate; HepG2, Jurkat, NIH/3T3, PC-12, Ramos, C6 and SH-SY5Y cell lysates; Mouse embryo, skin and lung tissue lysates; Human skin, lung and artery tissue lysates. IHC-P: Human lung carcinoma and breast carcinoma tissues.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP618Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40759 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆ **(3)**	1/5000. Detects a band of approximately 60 kDa (predicted molecular weight: 65 kDa).
IHC-P	★★★★☆ **(4)**	1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

Application notes Is unsuitable for Flow Cyt,ICC/IF or IP.

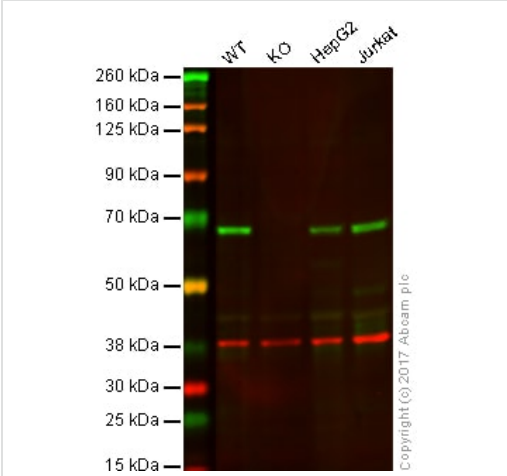
Target

Function Common SMAD (co-SMAD) is the coactivator and mediator of signal transduction by TGF-beta (transforming growth factor). Component of the heterotrimeric SMAD2/SMAD3-SMAD4 complex that forms in the nucleus and is required for the TGF-mediated signaling. Promotes binding of the SMAD2/SMAD4/FAST-1 complex to DNA and provides an activation function required for SMAD1 or SMAD2 to stimulate transcription. Component of the multimeric SMAD3/SMAD4/JUN/FOS complex which forms at the AP1 promoter site; required for syngernistic transcriptional activity in response to TGF-beta. May act as a tumor suppressor.

Involvement in disease Defects in SMAD4 are a cause of pancreatic cancer (PNCA) [MIM:260350].
Defects in SMAD4 are a cause of juvenile polyposis syndrome (JPS) [MIM:174900]; also known as juvenile intestinal polyposis (JIP). JPS is an autosomal dominant gastrointestinal hamartomatous polyposis syndrome in which patients are at risk for developing gastrointestinal cancers. The lesions are typified by a smooth histological appearance, predominant stroma, cystic spaces and lack of a smooth muscle core. Multiple juvenile polyps usually occur in a number of Mendelian disorders. Sometimes, these polyps occur without associated features as in JPS; here, polyps tend to occur in the large bowel and are associated with an increased risk of colon and other gastrointestinal cancers.
Defects in SMAD4 are a cause of juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome (JP/HHT) [MIM:175050]. JP/HHT syndrome phenotype consists of the coexistence of juvenile polyposis (JIP) and hereditary hemorrhagic telangiectasia (HHT) [MIM:187300] in a single individual. JIP and HHT are autosomal dominant disorders with distinct and non-overlapping clinical features. The former, an inherited gastrointestinal malignancy predisposition, is caused by mutations in SMAD4 or BMPR1A, and the latter is a vascular malformation disorder caused by mutations in ENG or ACVRL1. All four genes encode proteins involved in the transforming-growth-factor-signaling pathway. Although there are reports of patients and families with phenotypes of both disorders combined, the genetic etiology of this association is unknown.
Defects in SMAD4 may be a cause of colorectal cancer (CRC) [MIM:114500].

Sequence similarities	Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.
Domain	The MH1 domain is required for DNA binding. The MH2 domain is required for both homomeric and heteromeric interactions and for transcriptional regulation. Sufficient for nuclear import.
Post-translational modifications	Monoubiquitinated on Lys-519 by E3 ubiquitin-protein ligase TRIM33. Monoubiquitination hampers its ability to form a stable complex with activated SMAD2/3 resulting in inhibition of TGF-beta/BMP signaling cascade. Deubiqitination by USP9X restores its competence to mediate TGF-beta signaling.
Cellular localization	Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand. Migrates to the nucleus when complexed with R-SMAD.

Images

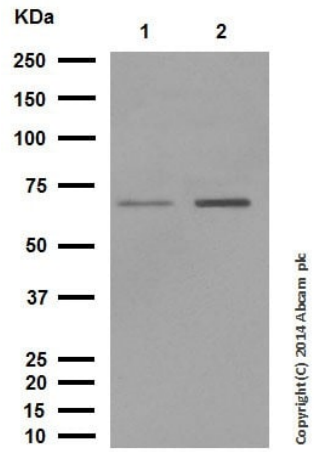


Western blot - Anti-Smad4 antibody [EP618Y] (ab40759)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: SMAD4 knockout HAP1 whole cell lysate (20 µg)
Lane 3: HepG2 whole cell lysate (20 µg)
Lane 4: Jurkat whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab40759 observed at 60 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab40759 was shown to specifically react with SMAD4 in wild type HAP1 cells. No band was observed when SMAD4 knockout HAP1 samples were used. Wild-type and SMAD4 knockout samples were subjected to SDS-PAGE. Ab40759 and **ab9484** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Smad4 antibody [EP618Y] (ab40759)

All lanes : Anti-Smad4 antibody [EP618Y] (ab40759) at 1/5000 dilution (purified)

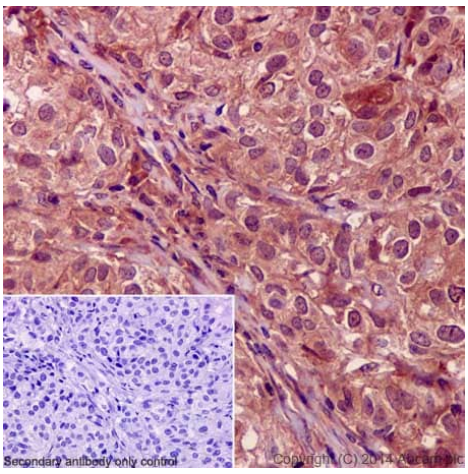
Lane 1 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) cell lysate
Lane 2 : Ramos (Human Burkitt's lymphoma cell line) cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

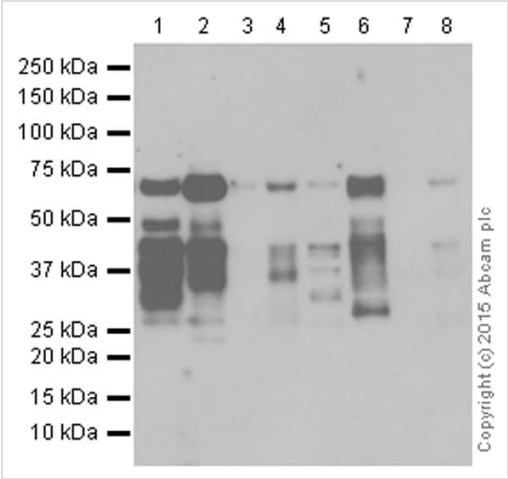
Predicted band size: 65 kDa
Observed band size: 60 kDa

Blocking and diluting buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad4 antibody [EP618Y] (ab40759)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling Smad4 with purified ab40759 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Western blot - Anti-Smad4 antibody [EP618Y] (ab40759)

All lanes : Anti-Smad4 antibody [EP618Y] (ab40759) at 1/5000 dilution

Lane 1 : NIH/3T3 (Mouse embyro fibroblast cell line) cell lysate
Lane 2 : Mouse embryo tissue lysate
Lane 3 : Mouse skin tissue lysate
Lane 4 : Mouse lung tissue lysate
Lane 5 : PC-12 (Rat adrenal gland pheochromocytoma cell line) cell lysate
Lane 6 : C6 cell lysate
Lane 7 : Rat skin tissue lysate
Lane 8 : Rat lung tissue lysate

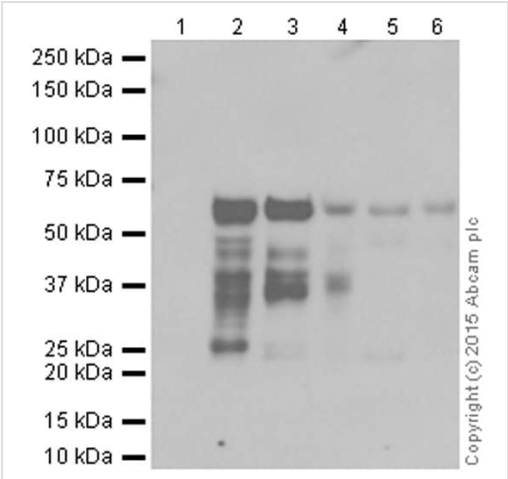
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 65 kDa
Observed band size: 60 kDa

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Smad4 antibody [EP618Y] (ab40759)

All lanes : Anti-Smad4 antibody [EP618Y] (ab40759) at 1/5000 dilution

Lane 1 : SW480 (Human colorectal adenocarcinoma cell line) cell lysate
Lane 2 : HepG2 (Human liver hepatocellular carcinoma cell line) cell lysate
Lane 3 : Jurkat (Human T cell leukemia cell line from peripheral blood) cell lysate
Lane 4 : Human skin tissue lysate
Lane 5 : Human lung tissue lysate
Lane 6 : Human artery tissue lysate

Lysates/proteins at 20 µg per lane.

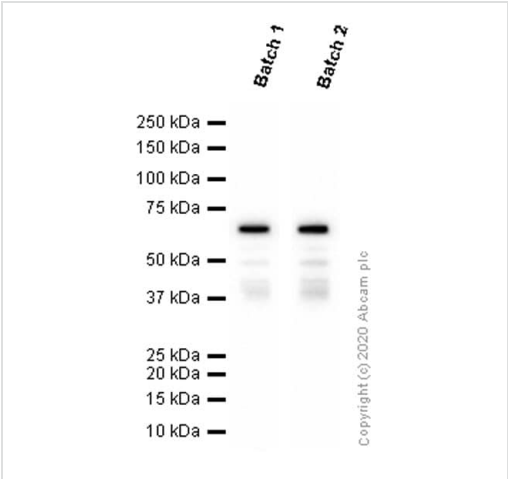
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 65 kDa
Observed band size: 60 kDa

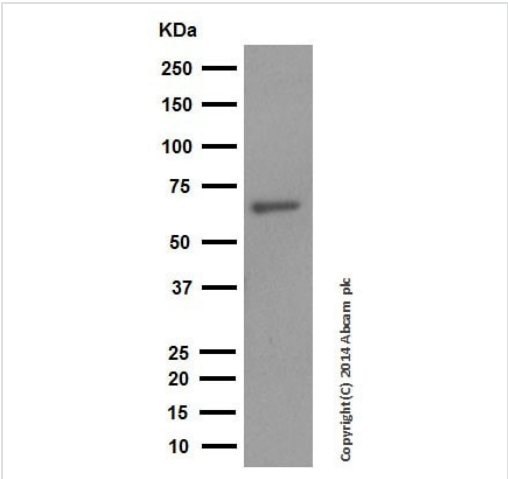
Exposure time: 30 seconds

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Smad4 antibody [EP618Y] (ab40759)

Different batches of ab40759 were tested on Ramos (Human Burkitt's lymphoma B lymphocyte) lysate at 0.7 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 60 kDa.



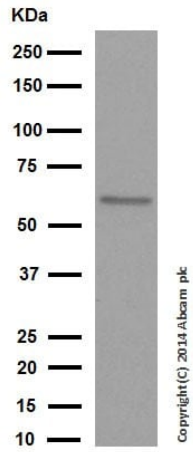
Western blot - Anti-Smad4 antibody [EP618Y] (ab40759)

Anti-Smad4 antibody [EP618Y] (ab40759) at 1/10000 dilution (purified) + NIH/3T3 (Mouse embyro fibroblast cell line) cell lysate at 20 µg

Secondary
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 65 kDa
Observed band size: 60 kDa

Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-Smad4 antibody [EP618Y] (ab40759)

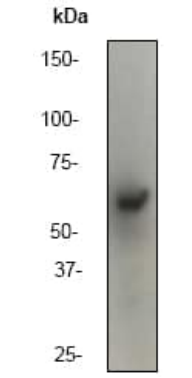
Anti-Smad4 antibody [EP618Y] (ab40759) at 1/5000 dilution (purified) + PC-12 (Rat adrenal gland pheochromocytoma cell line) cell lysate at 10 µg

Secondary

Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 65 kDa
Observed band size: 60 kDa

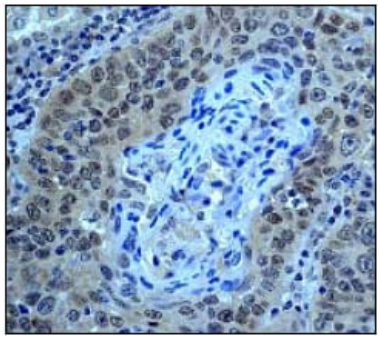
Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-Smad4 antibody [EP618Y] (ab40759)

Anti-Smad4 antibody [EP618Y] (ab40759) at 1/5000 dilution (unpurified) + SHSY5Y (Human neuroblastoma cell line from bone marrow) cell lysate at 10 µg

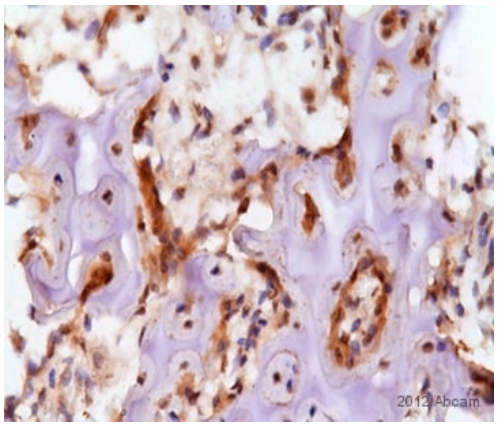
Predicted band size: 65 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad4 antibody [EP618Y] (ab40759)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung carcinoma tissue labelling Smad4 with unpurified ab40759 at a 1/100 dilution.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad4 antibody [EP618Y] (ab40759)

This image is courtesy of an anonymous Abreview

Unpurified ab40759 staining Smad4 in rat femur tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 1% BSA for 20 minutes at 22°C; antigen retrieval was by heat mediation in a citrate buffer pH6.0. Samples were incubated with primary antibody (1/200 in blocking buffer) for 2 hours at 20°C. An undiluted HRP-conjugated goat anti-rabbit IgG polyclonal (1/250) was used as the secondary antibody.

Anti-Smad4 antibody [EP618Y] (ab40759)

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