abcam

Product datasheet

Anti-Smad4 antibody ab110175

KO VALIDATED

4 References 3 Images

Overview

Product name	Anti-Smad4 antibody
Description	Rabbit polyclonal to Smad4
Host species	Rabbit
Tested applications	**Suitable for:** WB **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Sheep, Horse, Cow, Dog, Pig, Chimpanzee, Macaque monkey, Gorilla, Orangutan
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, A431, Jurkat, A549, HepG2, THP1 and Ramos whole cell lysates.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab110175 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		Use a concentration of 1 µg/ml. Detects a band of approximately 67 kDa (predicted molecular weight: 60 kDa).

Application notes Is unsuitable for ICC/IF.

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

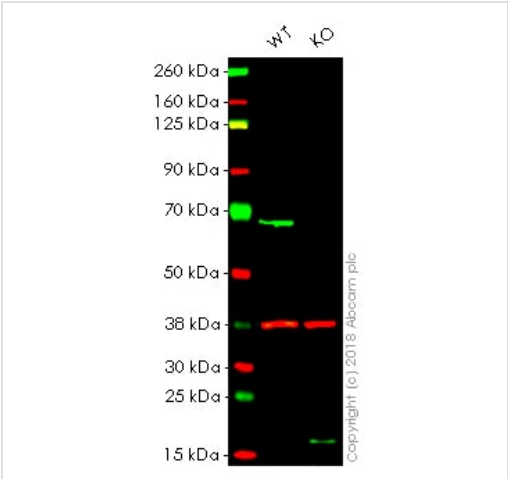
Belongs to the dwarfin/SMAD family.
Contains 1 MH1 (MAD homology 1) domain.
Contains 1 MH2 (MAD homology 2) domain.

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 (ab110175)

All lanes : Anti-Smad4 antibody (ab110175) at 1 µg

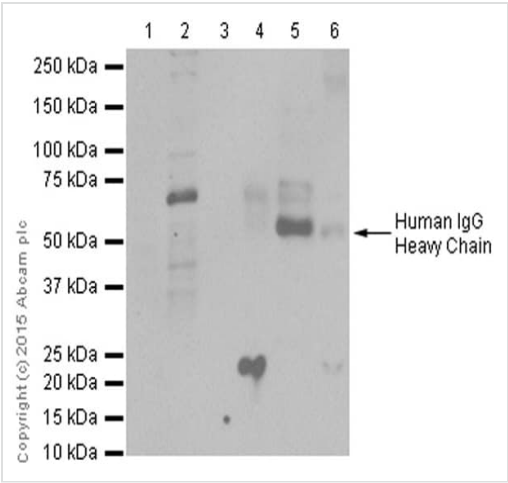
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : SMAD4 knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 60 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab110175 observed at 60 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab110175 was shown to recognize Smad4 in wild-type HAP1 cells as signal was lost at the expected MW in SMAD4 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and SMAD4 knockout samples were subjected to SDS-PAGE. Ab110175 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml 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 (ab110175)

All lanes : Anti-Smad4 antibody (ab110175) at 1/1000 dilution

Lane 1 : SW480 cell lysate
Lane 2 : HepG2 cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : Human skin tissue lysate
Lane 5 : Human lung tissue lysate
Lane 6 : Human artery tissue lysate

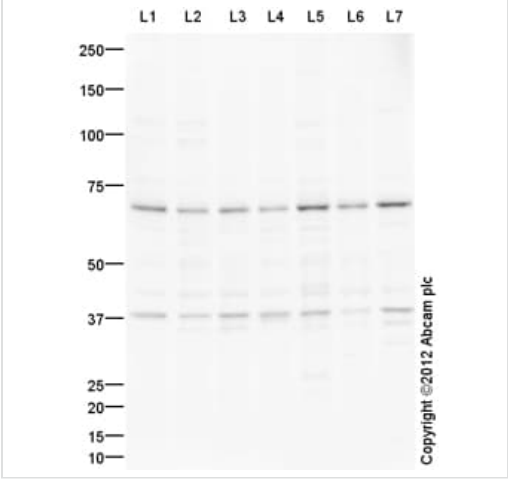
Lysates/proteins at 20 µg per lane.

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

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

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Smad4 antibody (ab110175)

All lanes : Anti-Smad4 antibody (ab110175) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 3 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
Lane 4 : A549 (Human lung adenocarcinoma epithelial cell line) Whole Cell Lysate
Lane 5 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 6 : THP1 (Human acute monocytic leukemia cell line) Whole

Cell Lysate
Lane 7 : Ramos (Human Burkitt's lymphoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 60 kDa
Observed band size: 67 kDa
Additional bands at: 37 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 4 minutes

The predicted molecular weight of Smad4 is 60 kDa (SwissProt), however we expect to observe a banding pattern around 70 kDa. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.

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