

Product datasheet

Anti-Smad4 antibody [EP618Y] α b40759

RabMAb

★★★★☆ 8 Abreviews 20 References 9 Images

Overview

Product name	Anti-Smad4 antibody [EP618Y]
Description	Rabbit monoclonal [EP618Y] to Smad4
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 (the amino acid sequence is considered to be commercially sensitive) corresponding to Human Smad4 aa 500 to the C-terminus (C terminal).
Positive control	WB: NIH/3T3, PC-12, Ramos and SH-SY5Y cell lysates. IHC-P: human lung carcinoma and breast carcinoma tissues.
General notes	This product is a recombinant rabbit monoclonal antibody.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

Produced using Abcam's RabMAb[®] technology. RabMAb[®] technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487.

Alternative versions available:

[Anti-Smad4 antibody \(HRP\) \[EP618Y\] \(ab195554\)](#)

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: 7.40 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

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	★★★★☆	1/5000. Detects a band of approximately 60 kDa (predicted molecular weight: 65 kDa). For unpurified use at 1/1000 - 1/5000.
IHC-P	★★★★☆	1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See protocols (link: http://www.abcam.com/protocols/ihc-antigen-retrieval-protocol). For unpurified use at 1/100 - 1/200.

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 synergistic 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.

Sequence similarities

Defects in SMAD4 may be a cause of colorectal cancer (CRC) [MIM:114500].

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.

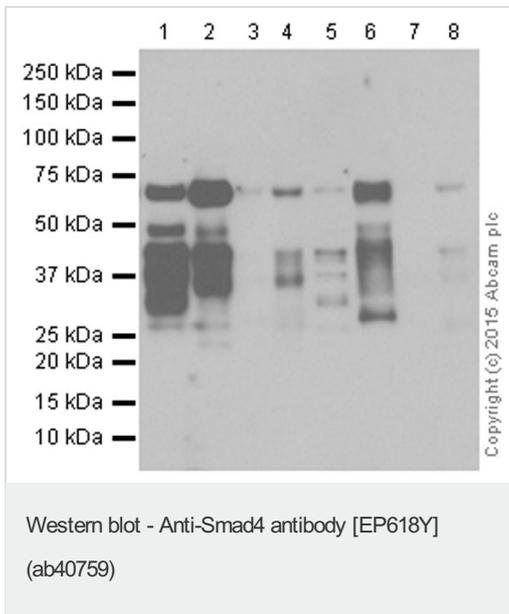
Post-translational modifications

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. Deubiquitination 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.

Anti-Smad4 antibody [EP618Y] images



All lanes : Anti-Smad4 antibody [EP618Y]

(ab40759) at 1/5000 dilution

Lane 1 : NIH/3T3 cell lysate

Lane 2 : Mouse embryo tissue lysate

Lane 3 : Mouse skin tissue lysate

Lane 4 : Mouse lung tissue lysate

Lane 5 : PC-12 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

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at

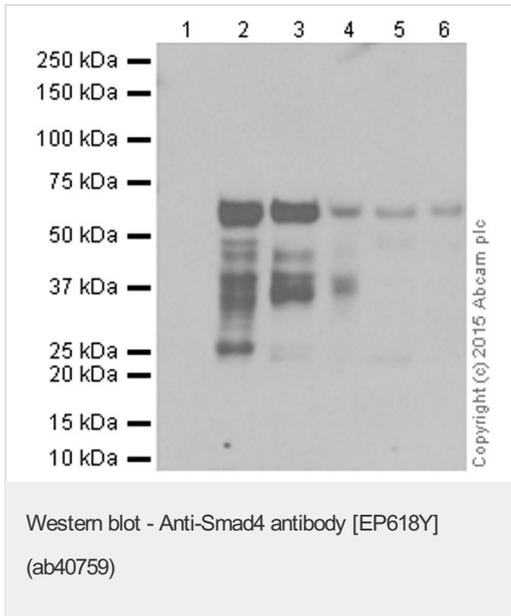
1/20000 dilution

Predicted band size : 65 kDa

Observed band size : 60 kDa

Exposure time : 3 minutes

Blocking and dilution buffer: 5% NFD/MTBST.



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

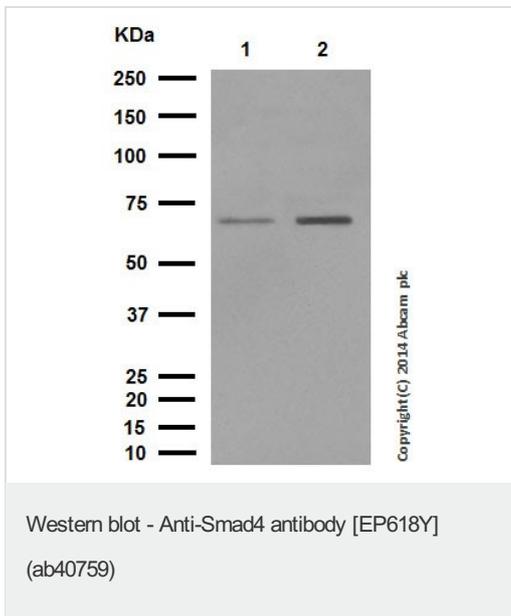
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size : 65 kDa

Observed band size : 60 kDa

Exposure time : 30 seconds

Blocking and dilution buffer: 5% NFDm/TBST.



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

Lane 1 : SH-SY5Y cell lysate

Lane 2 : Ramos cell lysate

Lysates/proteins at 10 µg per lane.

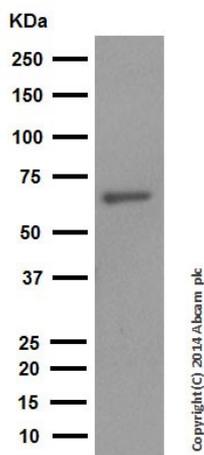
Secondary

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
1/10000 dilution (purified) + NIH/3T3 cell
lysate at 20 μ g

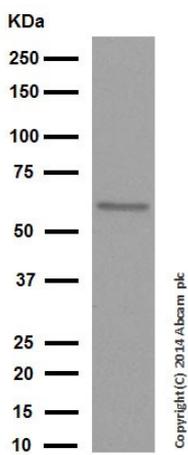
Secondary

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 cell lysate at
10 μ g

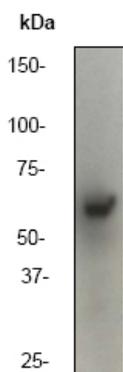
Secondary

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

Predicted band size : 65 kDa

Observed band size : 60 kDa

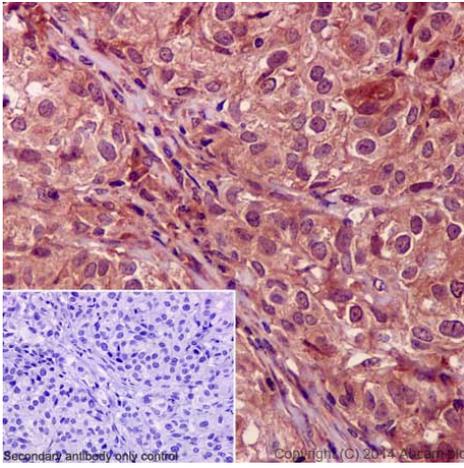
Blocking and diluting buffer: 5% NFDm/TBST.



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

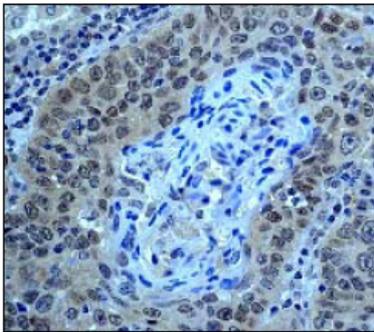
Anti-Smad4 antibody [EP618Y] (ab40759) at
1/5000 dilution (unpurified) + SHSY5Y cell
lysate at 10 μ g

Predicted band size : 65 kDa



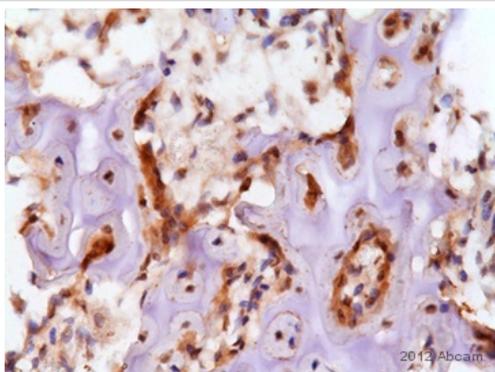
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling Smad4 with purified ab40759 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. [ab97051](#), a HRP-conjugated goat anti-rabbit IgG (H+L) was used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

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.

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



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.

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

This image is courtesy of an anonymous Abreview

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