

Product datasheet

Anti-Smad4 antibody [EP618Y] - BSA and Azide free
ab215968

KO VALIDATED Recombinant RabMAb

20 References 6 Images

Overview

Product name	Anti-Smad4 antibody [EP618Y] - BSA and Azide free
Description	Rabbit monoclonal [EP618Y] to Smad4 - BSA and Azide free
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 (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	Ab215968 is the carrier-free version of ab40759 . This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes.

Our [carrier-free formats](#) are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency.

Use our [conjugation kits](#) for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

ab215968 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm.

Maxpar® is a trademark of Fluidigm Canada Inc.

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](#).

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.

Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been “predicted to work with,” however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

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, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP618Y
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab215968** 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
-------------	-----------	-------

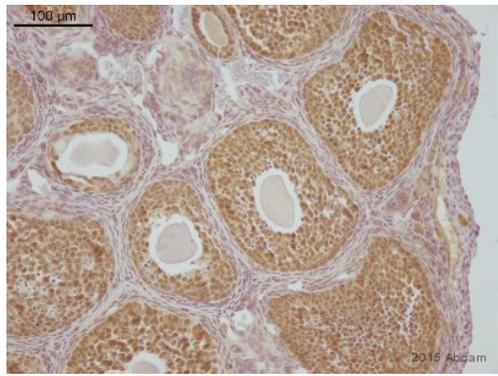
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 60 kDa (predicted molecular weight: 65 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval 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 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. 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. 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.

Images

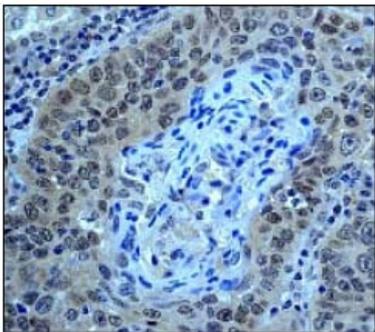


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad4 antibody [EP618Y] - BSA and Azide free (ab215968)
This image is courtesy of an anonymous Abreview.

4% PFA-fixed, paraffin-embedded postnatal day 21 mouse ovary tissue stained for Smad4 using [ab40759](#) at 1/500 dilution in immunohistochemical analysis.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab40759](#)).

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

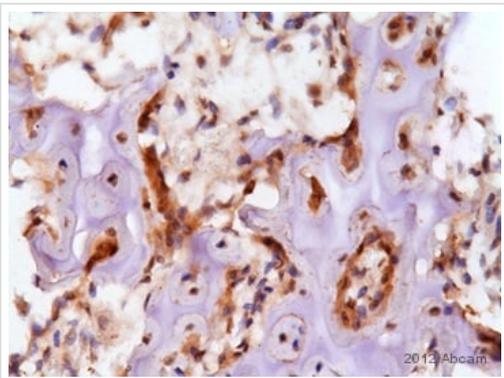


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad4 antibody [EP618Y] - BSA and Azide free (ab215968)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab40759](#)).

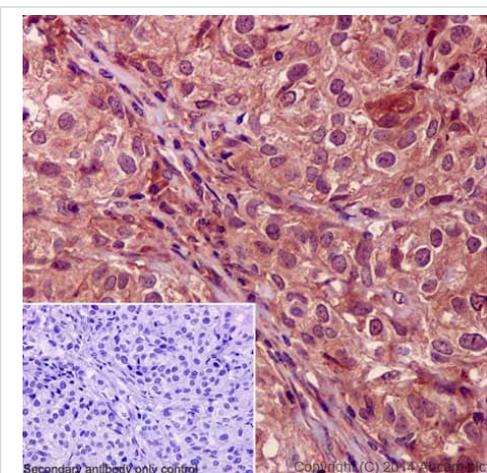
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad4 antibody [EP618Y] - BSA and Azide free (ab215968)
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.

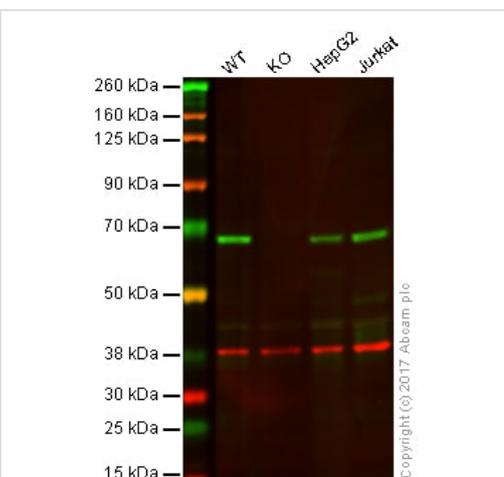
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab40759](#)).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad4 antibody [EP618Y] - BSA and Azide free (ab215968)

This IHC data was generated using the same anti-Smad4 antibody clone, EP618Y, in a different buffer formulation (cat# [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] - BSA and Azide free (ab215968)

This WB data was generated using the same anti-Smad4 antibody clone, EP618Y, in a different buffer formulation (cat# [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.

Why choose a recombinant antibody?



- Research with confidence**
Consistent and reproducible results
- Long-term and scalable supply**
Recombinant technology
- Success from the first experiment**
Confirmed specificity
- Ethical standards compliant**
Animal-free production

Anti-Smad4 antibody [EP618Y] - BSA and Azide free (ab215968)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors