abcam

Product datasheet

HRP Anti-Smad4 antibody [EP618Y] ab195554

KO VALIDATED Recombinant RabMAb

3 Images

Overview

Product name	HRP Anti-Smad4 antibody [EP618Y]
Description	HRP Rabbit monoclonal [EP618Y] to Smad4
Host species	Rabbit
Conjugation	HRP
Tested applications	**Suitable for:** WB **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: SH SY5Y whole cell lysate.
General notes	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. Store In the Dark.
Storage buffer	pH: 7.40 Preservative: 0.1% Proclin 300 Solution Constituents: 30% Glycerol (glycerin, glycerine), 1% BSA, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP618Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab195554 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 65 kDa (predicted molecular weight: 65 kDa).

Application notes Is unsuitable for IHC-P.

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

All lanes : HRP Anti-Smad4 antibody [EP618Y] (ab195554) at 1/5000 dilution

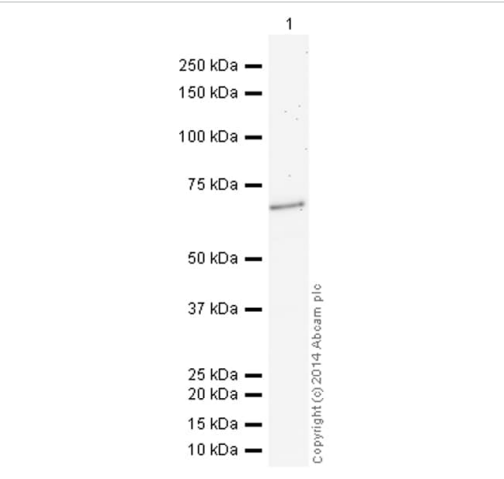
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : SMAD4 knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Exposure time: 12 minutes

ab195554 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. Ab195554 and **ab184095** (Mouse monoclonal [mAbcam 9484] to GAPDH - Loading Control (Alexa Fluor® 680) loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 dilution respectively. The loading control was imaged using the Licor Odyssey CLx prior to blots being developed with ECL technique.



Western blot - HRP Anti-Smad4 antibody [EP618Y] (ab195554)

HRP Anti-Smad4 antibody [EP618Y] (ab195554) at 1/5000 dilution + SHSY-5Y (Human neuroblastoma cell line) Whole Cell Lysate at 10 µg

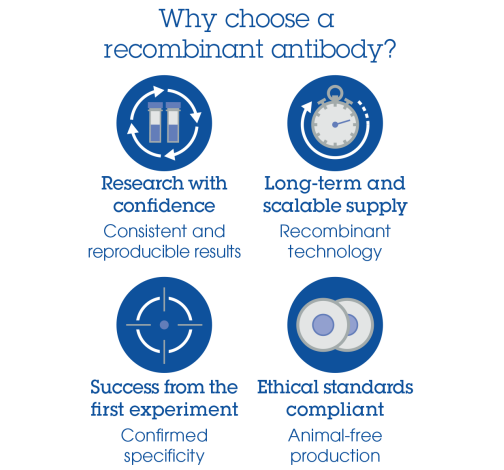
Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 20 minutes

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab195554 overnight at 4°C. Antibody binding was visualised using ECL development solution **ab133406**.



HRP Anti-Smad4 antibody [EP618Y] (ab195554)

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