# abcam

## Product datasheet

## Anti-Smad4 antibody [EP618Y] ab40759





重组 RabMAb

★★★★ 9 Abreviews 153 References 12 图像

概述

产品名称 Anti-Smad4抗体[EP618Y]

描述 兔单克隆抗体[EP618Y] to Smad4

宿主 Rabbit

经测试应用 适用于: WB, IHC-P

不适用于: Flow Cyt,ICC/IF or IP

种属反应性 与反应: Mouse, Rat, Human

免疫原 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)

阳性对照 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.

常规说明 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**.

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

存储溶液 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

纯**度** Protein A purified

**克隆** 单克隆

**克隆编号** EP618Y

**同种型** IgG

#### 应用

## The Abpromise quarantee Abpromise™承诺保证使用ab40759于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度:实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★☆(3)	1/5000. Detects a band of approximately 60 kDa (predicted molecular weight: 65 kDa).
IHC-P	<b>★★★★ ☆ (4)</b>	1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  See IHC antigen retrieval protocols.

应用说明

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

#### 靶标

#### 功能

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.

#### 疾病相关

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].

序列相似性 Belongs to the dwarfin/SMAD family.

Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.

结**构域** 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.

翻译后修饰 Monoubiquitinated on Lys-519 by E3 ubiquitin-protein ligase TRIM33. Monoubiquitination

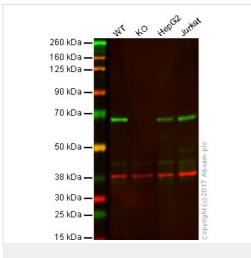
hampers its ability to form a stable complex with activated SMAD2/3 resulting in inhibition of TGF-beta/BMP signaling cascade. Deubiqitination by USP9X restores its competence to mediate

TGF-beta signaling.

细胞定位 Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand. Migrates to the nucleus when

complexed with R-SMAD.

#### 图片



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

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

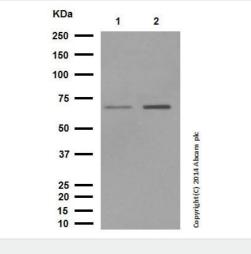
Lane 2: SMAD4 knockout HAP1 whole cell lysate (20 µg)

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 lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

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

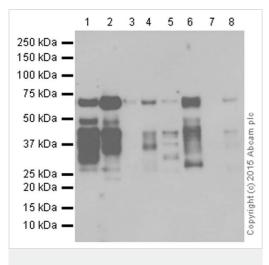
Blocking and diluting buffer: 5% NFDM/TBST.

Sorgandary antibody only control:

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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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. <a href="mailto:ab97051">ab97051</a>, 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.

1 2 3 4 5 6

250 kDa —
150 kDa —
100 kDa —
75 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
15 kDa —

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

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

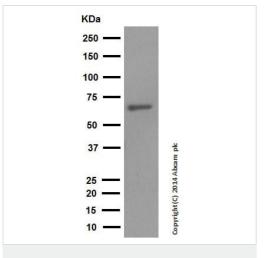
Exposure time: 30 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

250 kDa —
150 kDa —
100 kDa —
75 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
110 kDa —
110 kDa —
110 kDa —

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

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



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

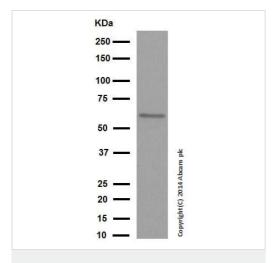
Anti-Smad4 antibody [EP618Y] (ab40759) at 1/10000 dilution (purified) + NIH/3T3 (Mouse embyro fibroblast cell line) cell lysate at 20 µg

## Secondary

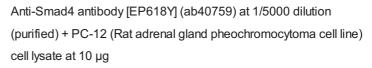
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

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

Blocking and diluting buffer: 5% NFDM/TBST.



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

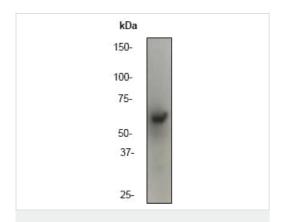


## Secondary

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

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

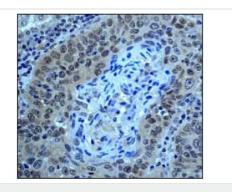
Blocking and diluting buffer: 5% NFDM/TBST.



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

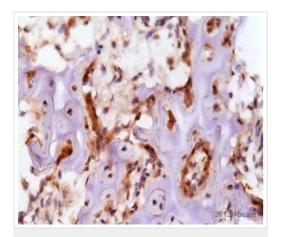
Predicted band size: 65 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

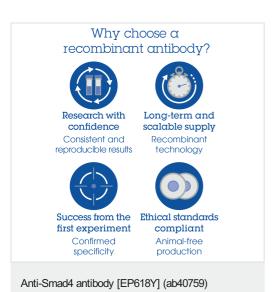
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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, paraffinembedded 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.



 $\textbf{Please note:} \ \ \textbf{All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"}$ 

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