abcam

ab230815

Anti-Smad4 antibody [EPR22589-112]

Recombinant | RabMAb | Advanced Validation | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR22589-112
Purification technique	Affinity purification Protein A
Concentration	0.556 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Mouse
Dilution info	1/30
Notes	-

Species	Human

Dilution info	1/30
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

ChIP

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

IHC-P

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

ChIC/CUT&RUN-seq

Tested

Species	Human

Dilution info	5 µg
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information SMAD4

Function

In muscle physiology, plays a central role in the balance between atrophy and hypertrophy. When recruited by MSTN, promotes atrophy response via phosphorylated SMAD2/4. MSTN decrease causes SMAD4 release and subsequent recruitment by the BMP pathway to promote hypertrophy via phosphorylated SMAD1/5/8. Acts synergistically with SMAD1 and YY1 in bone morphogenetic protein (BMP)-mediated cardiac-specific gene expression. Binds to SMAD binding elements (SBEs) (5'-GTCT/AGAC-3') within BMP response element (BMPRE) of cardiac activating regions (By similarity). 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 (PubMed:25514493). 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. Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C

Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Smad4 also known as DPC4 or MADH4 is a central protein in the TGF-beta signaling pathway with a molecular mass of approximately 60 kDa. It acts as a signal transducer that facilitates communication from the cell surface to the nucleus. Smad4 is broadly expressed in various tissues playing an important role in the regulation of cellular processes. It forms a complex with receptor-regulated Smads (R-Smads) to translocate to the nucleus where it influences gene transcription.
Biological function summary	Smad4 influences cell proliferation differentiation and apoptosis by mediating signals from TGF-beta cytokines. It is part of the Smad protein family acting as a transcriptional controller. Upon TGF-beta receptor activation Smad4 forms complexes with Smad2 and Smad3 translocating to the nucleus to regulate genes imperative for cellular homeostasis. Its role in cell cycle regulation underlines its contribution to normal cellular functions and its potential involvement in disorders.
Pathways	Smad4 operates within the TGF-beta pathway linking extracellular signals to nuclear transcription alterations. It participates in the regulation of epithelial-mesenchymal transition (EMT) a process important for development and tumor progression. In these pathways Smad4 interacts closely with Smad2 and Smad3 orchestrating various cellular responses to external stimuli through transcriptional management.

Associated diseases and disorders	Smad4 is highly related to cancer and juvenile polyposis syndrome. Mutations or deletions in Smad4 disrupt its function contributing to the progression of pancreatic cancer and colorectal cancer among others. Within these contexts Smad4 connects strongly to other proteins like p21 and cyclin-dependent kinase inhibitors which are important in cell cycle arrest and impede tumor growth.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

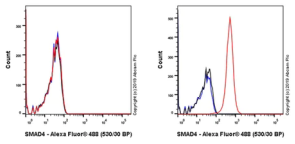
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

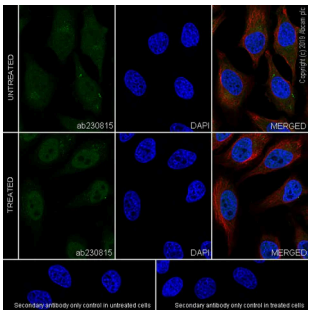
11 product images



Flow Cytometry (Intracellular) - Anti-Smad4 antibody [EPR22589-112] (ab230815)

Smad4 Flow Cytometry (Intracellular) staining using rabbit Anti-Smad4 antibody

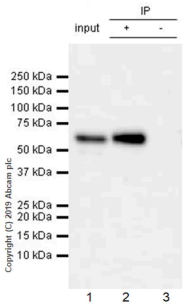
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed Smad4 KO HAP1 (Smad4 knockout human chronic myelogenous leukemia near-haploid cell line, Left) / WT HAP1 (human chronic myelogenous leukemia near-haploid cell line, Right) cell line labeling Smad4 with ab230815 at 1/500 (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor$^{ï¿½}$ 488, ab150077) was used as secondary antibody at 1/2000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-Smad4 antibody [EPR22589-112] (ab230815)

Smad4 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad4 antibody

Immunofluorescent analysis of HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Smad4 with ab230815 at a 1/100 dilution (green). Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Confocal image showing mainly nuclear staining in HeLa cell line treated with TGF-beta (10ng/ml) for 1 h. AlexaFluor®488 Goat anti-Rabbit (ab150077) was used secondary antibody at 1/1000 dilution, this was also used on its own as a control. DAPI was used as a nuclear counterstain (blue). Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594, ab195889) was used as a counterstain at 1/200 dilution.



Immunoprecipitation - Anti-Smad4 antibody [EPR22589-112] (ab230815)

Smad4 was immunoprecipitated from 0.35mg of HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate with ab230815 at 1/30 dilution. Western Blot was performed from the immunoprecipitate using ab230815 at 1/1000 dilution (0.5 µg/ml).
VeriBlot for IP Detection Reagent (HRP) (ab131366), was used as secondary antibody at 1/5000 dilution.

Lane 1: HeLa whole cell lysate 10µg (Input).

Lane 2: ab230815 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab230815 in HeLa whole cell lysate

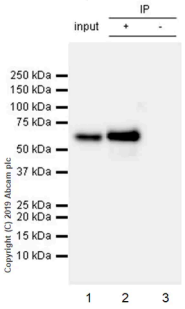
Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 30 seconds

Lysate was freshly prepared and IP test was done immediately to avoid protein degradation. Lysate incubation time was shortened from overnight to 2h.

All lanes:
Immunoprecipitation - Anti-Smad4 antibody [EPR22589-112] (ab230815)

Predicted band size: 60 kDa



Immunoprecipitation - Anti-Smad4 antibody [EPR22589-112] (ab230815)

Smad4 was immunoprecipitated from 0.35mg of NIH/3T3 (mouse embryonic fibroblast) whole cell lysate with ab230815 at 1/30 dilution. Western Blot was performed from the immunoprecipitate using ab230815 at 1/1000 dilution (0.5 µg/ml).
VeriBlot for IP Detection Reagent (HRP) (ab131366), was used as secondary antibody at 1/5000 dilution.

Lane 1: NIH/3T3 whole cell lysate 10µg (Input).

Lane 2: ab230815 IP in NIH/3T3 whole cell lysate

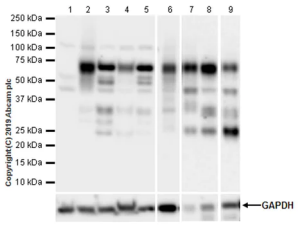
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab230815 in NIH/3T3 whole cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 10 seconds

Lysate was freshly prepared and IP test was done immediately to avoid protein degradation. Lysate incubation time was shortened from overnight to 2h.
All lanes:
Immunoprecipitation - Anti-Smad4 antibody [EPR22589-112] (ab230815)

Predicted band size: 60 kDa



Western blot - Anti-Smad4 antibody [EPR22589-112] (ab230815)

Smad4 Western blot staining using rabbit Anti-Smad4 antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
Exposure Time: Lanes 1-6: 15 secs; Lanes 7-8: 114 secs; Lane 9: 3 mins.

ab230815 was shown to specifically react with Smad4 in wild-type HAP1 cells as signal was lost in Smad4 knockout cells. Wild-type and Smad4 knockout samples were subjected to SDS-PAGE. ab230815 and ab181602 (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Smad4 antibody [EPR22589-112] (ab230815) at 1/1000 dilution

Lane 1:
Smad4 knockout HAP1 whole cell lysate at 20 µg

Lane 2:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 3:
HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Lane 4:
HCT116 (human colorectal carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 5:
HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 6:
Human fetal lung tissue lysate at 20 µg

Lane 7:
Mouse embryo tissue lysate at 20 µg

Lane 8:
Mouse lung tissue lysate at 20 µg

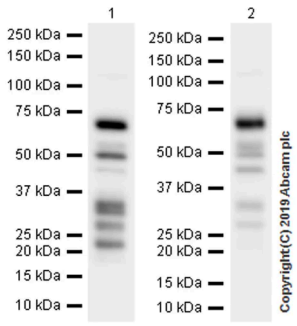
Lane 9:
Rat lung tissue lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 60 kDa

Observed band size: 60 kDa



Western blot - Anti-Smad4 antibody [EPR22589-112] (ab230815)

Blocking/Dilution buffer: 5% NFDM/TBST.
Exposure time: Lane 1: 15 secs; Lane 2: 7.75 secs.

All lanes:
Western blot - Anti-Smad4 antibody [EPR22589-112] (ab230815) at 1/1000 dilution

Lane 1:
HEK-293 (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

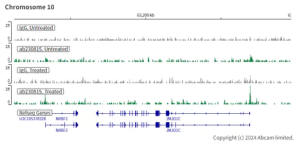
Lane 2:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 60 kDa

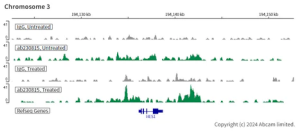
Observed band size: 60 kDa



ChIC/CUT&RUN sequencing - Anti-Smad4 antibody [EPR22589-112] (ab230815)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human epithelial cell line from cervix adenocarcinoma) cells treated with hTGF-β1 (10 ng/mL 1 h) and 5 µg of ab230815 [EPR22589-112]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control ab172730 is also shown.

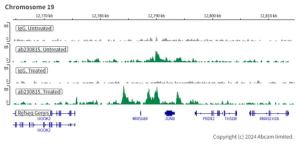
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



ChIC/CUT&RUN sequencing - Anti-Smad4 antibody [EPR22589-112] (ab230815)

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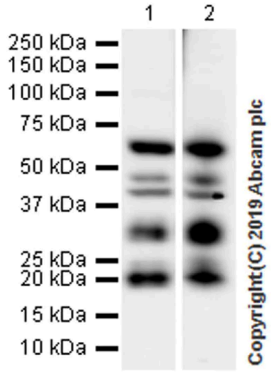


ChIC/CUT&RUN sequencing - Anti-Smad4 antibody [EPR22589-112] (ab230815)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human epithelial cell line from cervix adenocarcinoma) cells treated with hTGF-β1 (10

ng/mL 1 h) and 5 µg of ab230815 [EPR22589-112]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control ab172730 is also shown.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-Smad4 antibody [EPR22589-112] (ab230815)

Smad4 Western blot staining using rabbit Anti-Smad4 antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-Smad4 antibody [EPR22589-112] (ab230815) at 1/1000 dilution

Lane 1:
RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 2:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 60 kDa

Exposure time: 48s

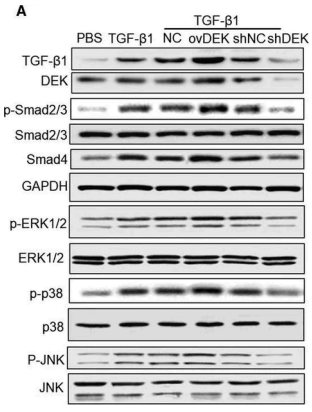


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Western blot - Anti-Smad4 antibody [EPR22589-112] (ab230815)

Smad4 Western blot staining using rabbit Anti-Smad4 antibody

Smad4 western blot using anti-Smad4 antibody [EPR22589-112] ab230815. Publication image and figure legend from Song, Y., Wang, Z., et al., 2020, J Cell Mol Med, PubMed 33124760.

ab230815 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab230815 please see the product overview.

Effects of DEK shRNA on Smad, MAPK and PI3K/AKT signalling pathways in BEAS-2B cells. (A-E) The expressions of TGF-β, DEK mad2/3 and Smad4, ERK1/2, p38, JNK, and PI3K, AKT, mTOR were detected by Western blot. The relative density of each protein was calculated. Data were shown as mean ± SD (n = 3). NC, negative control cells; ovDEK, cells transfected with DEK; shNC, cells transfected with scrambled shRNA; shDEK, cells transfected with DEK shRNA. *p < 0.05, vs control group. #p < 0.05, ovDEK vs TGF-β1 + NC group; shDEK vs TGF-β1 + shNC group

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