abcam

ab40771

Anti-SMAD5 antibody [EP619Y]

Recombinant | RabMAb | 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	EP619Y
Purification technique	Affinity purification Protein A
Concentration	0.552 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Dot

Not recommended

Species	Human, Mouse, Rat, African green monkey
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000 - 1/5000
Notes	-

Species	African green monkey
Dilution info	1/1000 - 1/5000
Notes	-

Species	Human
Dilution info	1/1000 - 1/5000
Notes	-

Expected

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

ICC/IF

Tested

Species	Human
Dilution info	1/50 - 1/100
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Rat
Dilution info	1/30 - 1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Species	Human
Dilution info	1/30 - 1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, African green monkey
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/50
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Expected

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

Target data

See full target information SMAD5

Function	Transcriptional regulator that plays a role in various cellular processes including embryonic development, cell differentiation, angiogenesis and tissue homeostasis (PubMed:12064918, PubMed:16516194). Upon BMP ligand binding to their receptors at the cell surface, is phosphorylated by activated type I BMP receptors (BMPRIs) and associates with SMAD4 to form a heteromeric complex which translocates into the nucleus acting as transcription factor (PubMed:9442019). In turn, the hetero-trimeric complex recognizes cis-regulatory elements containing Smad Binding Elements (SBEs) to modulate the outcome of the signaling network (PubMed:33510867). Non-phosphorylated SMAD5 has a cytoplasmic role in energy metabolism regulation by promoting mitochondrial respiration and glycolysis in response to cytoplasmic pH changes (PubMed:28675158). Mechanistically, interacts with hexokinase 1/HK1 and thereby accelerates glycolysis (PubMed:28675158).

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	SMAD5 also known as Mothers Against Decapentaplegic Homolog 5 is a protein involved in the signal transduction regulated by the transforming growth factor-beta (TGF-beta) superfamily of cytokines. This protein has a molecular mass of approximately 52 kDa and is ubiquitously expressed across various tissues. Mechanically SMAD5 functions as an intracellular mediator that translocates from the cytoplasm to the nucleus upon activation to regulate transcription. It participates actively in transmitting signals from surface receptors into the nucleus influencing gene expression.
Biological function summary	SMAD5 plays a central role in the bone morphogenetic protein (BMP) signaling pathway which is critical for embryonic development and tissue homeostasis. It forms a complex with receptor-regulated SMADs (R-SMADs) and co-SMAD (SMAD4) upon phosphorylation by BMP type I receptors. This complex then moves to the nucleus where it regulates expression of target genes. Through this mechanism SMAD5 impacts processes such as bone formation differentiation and cellular differentiation.
Pathways	Several key cellular signaling processes involve SMAD5. Primarily it contributes to the BMP pathway which is integral to skeletal development and repair. SMAD5 interacts with other SMAD proteins like SMAD1 and SMAD8 which all act downstream of the BMP receptors. Additionally SMAD5 participates in crosstalk with the TGF-beta signaling pathway enabling the fine-tuning of cellular responses to a variety of external cues. This connection places SMAD5 within a network of signaling events important for regulating different cell functions and maintaining cellular homeostasis.
Associated diseases and disorders	SMAD5's function has links to abnormal bone and cartilage development such as in fibrodysplasia ossificans progressiva (FOP) and pulmonary hypertension (PH). These conditions occur due to dysregulation within the BMP signaling pathway. For example mutations in SMAD5 or its regulatory proteins can cause inappropriate signal propagation leading to ossification in FOP or altered vascular remodeling in PH. The interplay between SMAD5 and SMAD4 another key player in these pathways highlights the complex nature of SMAD-related pathologies.

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

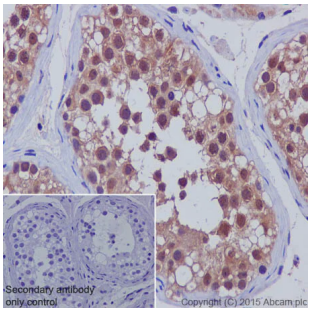
We do not recommend this combination. It is not covered by our product promise.

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.

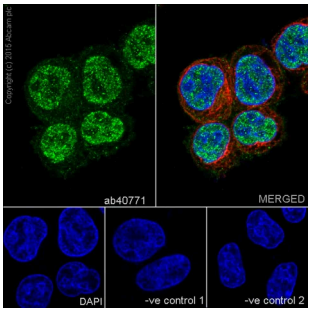
9 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD5 antibody [EP619Y] (ab40771)

SMAD5 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-SMAD5 antibody

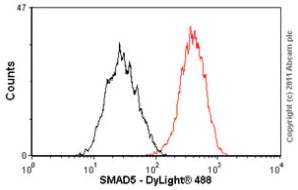
Immunohistochemical staining of paraffin embedded human testis with purified ab40771 at a working dilution of 1/50. The secondary antibody used is ab97051, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunocytochemistry/ Immunofluorescence - Anti-SMAD5 antibody [EP619Y] (ab40771)

SMAD5 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SMAD5 antibody

Immunofluorescence staining of HeLa cells with purified ab40771 at a working dilution of 1/100, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab40771 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.

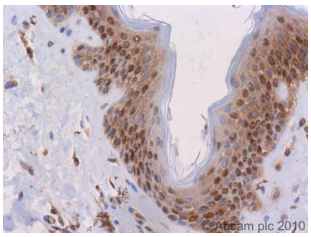


Flow Cytometry (Intracellular) - Anti-SMAD5 antibody [EP619Y] (ab40771)

SMAD5 Flow Cytometry (Intracellular) staining using rabbit Anti-SMAD5 antibody

Overlay histogram showing HEK293 cells stained with unpurified ab40771 (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab40771, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500

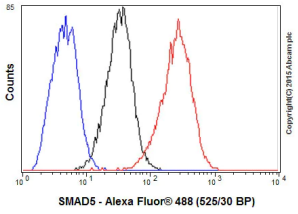
dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal IgG (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HEK293 cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD5 antibody [EP619Y] (ab40771)

SMAD5 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-SMAD5 antibody

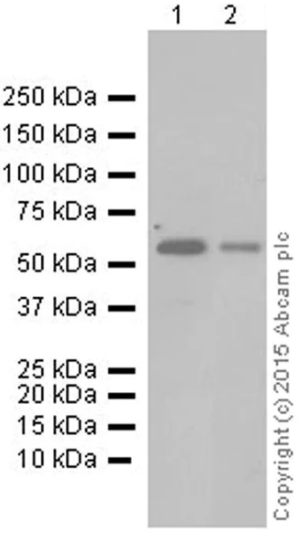
Unpurified ab40771 (4µg/ml) staining SMAD5 in human skin using an automated system (DAKO Autostainer Plus). Using this protocol there is strong staining of nuclear/cytoplasmic compartments within the stratum granulosum.
Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer EDTA pH 9.0 in a DAKO PT link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.



Flow Cytometry (Intracellular) - Anti-SMAD5 antibody [EP619Y] (ab40771)

SMAD5 Flow Cytometry (Intracellular) staining using rabbit Anti-SMAD5 antibody

Overlay histogram showing PC-12 cells fixed in 4% PFA and stained with purified ab40771 at a dilution of 1/100 (red line). The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit at a dilution of 1/500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).



Western blot - Anti-SMAD5 antibody [EP619Y] (ab40771)

SMAD5 Western blot staining using rabbit Anti-SMAD5 antibody

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-SMAD5 antibody [EP619Y] (ab40771) at 1/5000 dilution

Lane 1:
HEK293 whole cell lysate at 10 µg

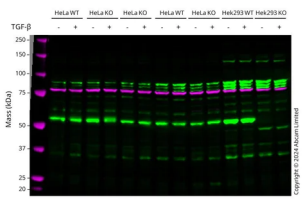
Lane 2:
COS-1 whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 52 kDa

Observed band size: 52 kDa



Western blot - Anti-SMAD5 antibody [EP619Y] (ab40771)

SMAD5 Western blot staining using rabbit Anti-SMAD5 antibody

Anti-SMAD5 antibody [EP619Y] (ab40771) staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab40771 was shown to bind specifically to SMAD5. A band was observed at 52 kDa in wild-type HeLa cell lysates with no signal observed at this size in SMAD5 knockout cell line. To generate this image, wild-type and SMAD5 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-SMAD5 antibody [EP619Y] (ab40771) at 1/1000 dilution

Lane 1:
Wild-type HeLa Vehicle Control TGF-beta (0 ng/mL, 30 min) cell lysate at 20 µg

Lane 2:
Wild-type HeLa Treated TGF-beta (10 ng/mL, 30 min) cell lysate at 20 µg

Lane 3:
SMAD1 knockout HeLa Vehicle Control TGF-beta (0 ng/mL, 30 min), ab265400 cell lysate at 20 µg

Lane 4:
SMAD1 knockout HeLa Treated TGF-beta (10 ng/mL, 30 min), ab265400 cell lysate at 20 µg

Lane 5:
SMAD2 knockout HeLa Vehicle Control TGF-beta (0 ng/mL, 30 min), ab255430 cell lysate at 20 µg

Lane 6:
SMAD2 knockout HeLa Treated TGF-beta (10 ng/mL, 30 min), ab255430 cell lysate at 20 µg

Lane 7:
Wild-type HeLa Vehicle Control TGF-beta (0 ng/mL, 30 min), ab255448 cell lysate at 20 µg

Lane 8:
Wild-type HeLa Treated TGF-beta (10 ng/mL, 30 min), ab255448 cell lysate at 20 µg

Lane 9:
SMAD3 knockout HeLa Vehicle Control TGF-beta (0 ng/mL, 30 min), ab255431 cell lysate at 20 µg

Lane 10:
SMAD3 knockout HeLa Treated TGF-beta (10 ng/mL, 30 min), ab255431 cell lysate at 20 µg

Lane 11:
Wild-type HEK293 Vehicle Control TGF-beta (0 ng/mL, 30 min), ab259776 cell lysate at 20 µg

Lane 12:
Wild-type HEK293 Treated TGF-beta (10 ng/mL, 30 min), ab259776 cell lysate at 20 µg

Lane 13:
SMAD5 knockout HEK293 Vehicle Control TGF-beta (0 ng/mL, 30 min), ab269470 cell lysate at 20 µg

Lane 14:

SMAD5 knockout HEK293 Treated TGF-beta (10 ng/mL, 30 min), ab269470 cell lysate at 20 µg

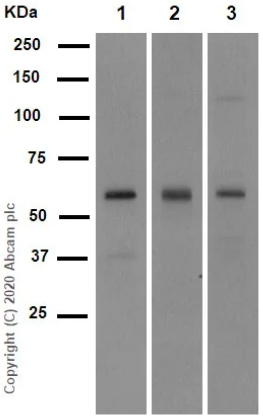
Secondary

Lanes 1 - 14:
Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution

Lanes 1 - 14:
Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 52 kDa



Western blot - Anti-SMAD5 antibody [EP619Y] (ab40771)

SMAD5 Western blot staining using rabbit Anti-SMAD5 antibody

Blocking and dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-SMAD5 antibody [EP619Y] (ab40771) at 1/1000 dilution

Lane 1:
3T3-L1 (Mouse embryonic fibroblast) lysate at 20 µg

Lane 2:
Neuro-2a (Mouse neuroblastoma neuroblast) at 20 µg

Lane 3:
F9 (Mouse embryonal carcinoma epithelial cell) lysate at 20 µg

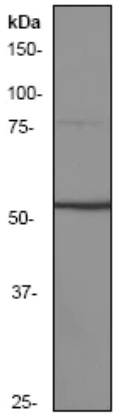
Secondary

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

Predicted band size: 52 kDa

Observed band size: 52 kDa

Exposure time: 3min



Western blot - Anti-SMAD5 antibody [EP619Y] (ab40771)

All lanes:
Western blot - Anti-SMAD5 antibody [EP619Y] (ab40771) at 1/1000 dilution

All lanes:
Cos-1 cell lysate at 10 µg

Predicted band size: 52 kDa

Observed band size: 52 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.