abcam

Product datasheet

Anti-SMAD5 antibody [EP619Y] ab40771

Recombinant RabMAb

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Overview

Product name	Anti-SMAD5 antibody [EP619Y]
Description	Rabbit monoclonal [EP619Y] to SMAD5
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, WB, ICC/IF, Dot blot
Species reactivity	**Reacts with:** Mouse, Rat, Human, African green monkey
Immunogen	Synthetic peptide within Human SMAD5 aa 200-300. The exact sequence is proprietary.
Positive control	WB: HEK293 and Cos-1 whole cell lysate. Flow Cyt (intra): PC-12 and HEK293 cells. ICC/IF: HeLa cells. IHC-P: Human testis tissue and human skin tissue.
General notes	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**.

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.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP619Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40771 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
Flow Cyt (Intra)		1/30 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★★ (1)	1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★★ (2)	1/1000 - 1/5000. Detects a band of approximately 52 kDa (predicted molecular weight: 52 kDa).
ICC/IF	★★★★★ (1)	1/50 - 1/100.
Dot blot		Use at an assay dependent concentration.

Target

Function Transcriptional modulator activated by BMP (bone morphogenetic proteins) type 1 receptor kinase. SMAD5 is a receptor-regulated SMAD (R-SMAD).

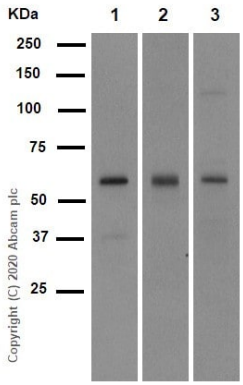
Tissue specificity Ubiquitous.

Sequence similarities Belongs to the dwarfin/SMAD family.
Contains 1 MH1 (MAD homology 1) domain.
Contains 1 MH2 (MAD homology 2) domain.

Post-translational modifications Phosphorylated on serine by BMP (bone morphogenetic proteins) type 1 receptor kinase. Ubiquitin-mediated proteolysis by SMAD-specific E3 ubiquitin ligase SMURF1.

Cellular localization Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand. Migrates to the nucleus when complexed with SMAD4.

Images



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

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

Lane 1 : 3T3-L1 (Mouse embryonic fibroblast) lysate
Lane 2 : Neuro-2a (Mouse neuroblastoma neuroblast)
Lane 3 : F9 (Mouse embryonal carcinoma epithelial cell) lysate

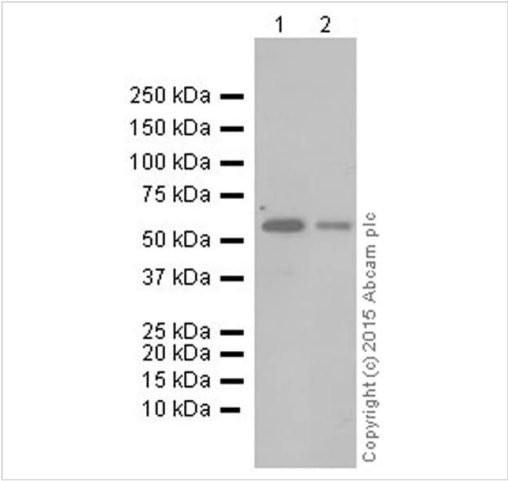
Lysates/proteins at 20 µg per lane.

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: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST



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

All lanes : Anti-SMAD5 antibody [EP619Y] (ab40771) at 1/5000 dilution (purified)

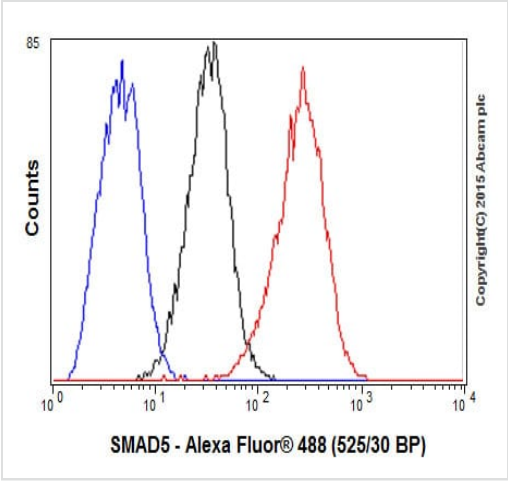
Lane 1 : HEK293 whole cell lysate
Lane 2 : COS-1 whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution (HRP goat anti-rabbit IgG (H+L))

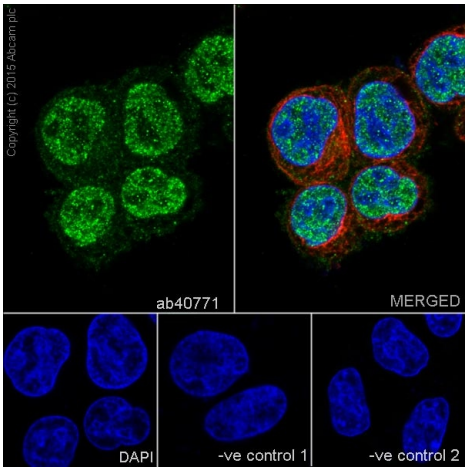
Predicted band size: 52 kDa
Observed band size: 52 kDa

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST



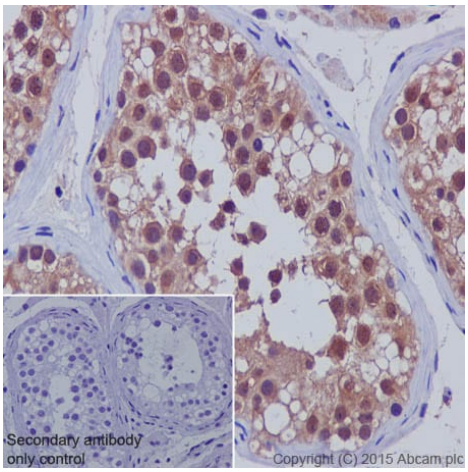
Flow Cytometry (Intracellular) - Anti-SMAD5 antibody [EP619Y] (ab40771)

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).



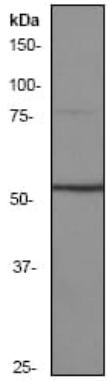
Immunocytochemistry/ Immunofluorescence - Anti-SMAD5 antibody [EP619Y] (ab40771)

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.



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

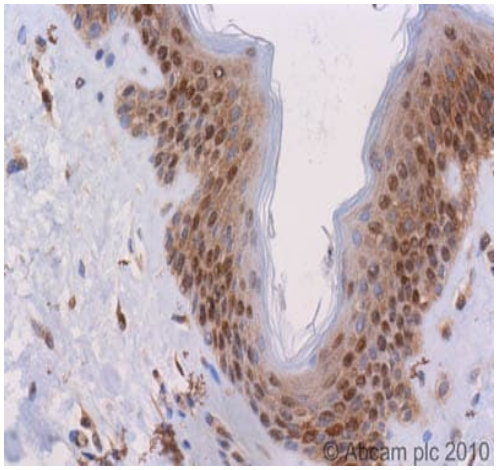
Immunohistochemical staining of paraffin embedded human testis with purified ab40771 at a working dilution of 1/50. The secondary antibody used is **ab97051**, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA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



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

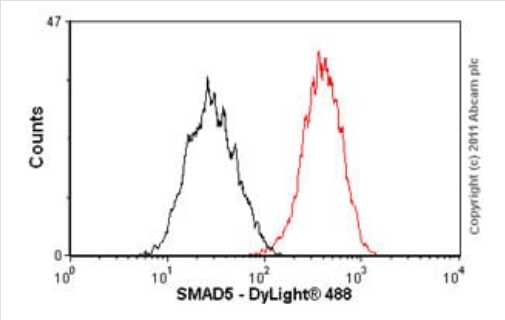
Anti-SMAD5 antibody [EP619Y] (ab40771) at 1/1000 dilution (unpurified) + Cos-1 cell lysate at 10 µg

Predicted band size: 52 kDa
Observed band size: 52 kDa



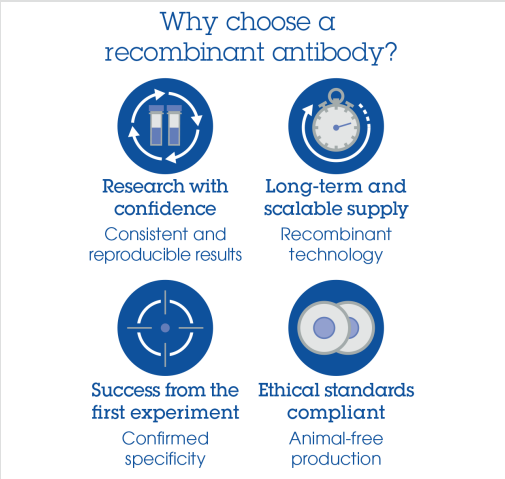
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD5 antibody [EP619Y] (ab40771)

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)

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.



Anti-SMAD5 antibody [EP619Y] (ab40771)

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