

Product datasheet

Anti-SMAD5 antibody [EP619Y] ab40771

リコンビナント RabMAb®

★★★★★ 2 Abreviews 12 References 画像数 7

製品の概要

製品名	Anti-SMAD5 antibody [EP619Y]
製品の詳細	Rabbit monoclonal [EP619Y] to SMAD5
由来種	Rabbit
アプリケーション	適用あり: IHC-P, WB, Flow Cyt, ICC/IF
種交差性	交差種: Rat, Human, African green monkey 交差が予測される動物種: Mouse
免疫原	Synthetic peptide within Human SMAD5 aa 200-300. The exact sequence is proprietary.
ポジティブ・コントロール	WB: HEK293 and Cos-1 whole cell lysate. Flow Cyt: PC-12 and HEK293 cells. ICC/IF: HeLa cells. IHC-P: Human testis tissue and human skin tissue.
特記事項	

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

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
精製度	Protein A purified

ポリモノ	モノクローナル
クローン名	EP619Y
アイソタイプ	IgG

アプリケーション

Our [Abpromise guarantee](#) covers the use of **ab40771** in the following tested applications.

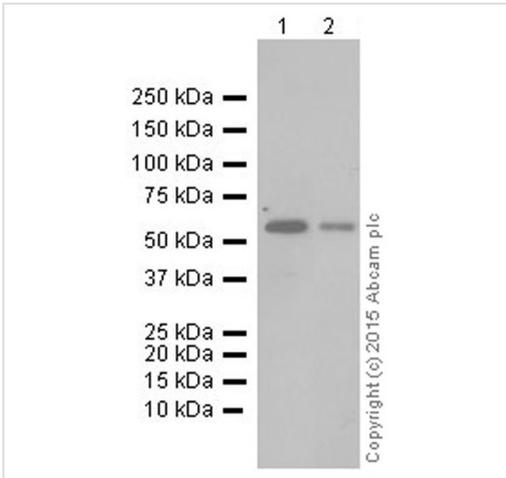
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

アプリケーション	Abreviews	特記事項
IHC-P		1/50.
WB	★★★★★	1/1000 - 1/5000. Detects a band of approximately 52 kDa (predicted molecular weight: 52 kDa).
Flow Cyt		1/30 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/50 - 1/100.

ターゲット情報

機能	Transcriptional modulator activated by BMP (bone morphogenetic proteins) type 1 receptor kinase. SMAD5 is a receptor-regulated SMAD (R-SMAD).
組織特異性	Ubiquitous.
配列類似性	Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.
翻訳後修飾	Phosphorylated on serine by BMP (bone morphogenetic proteins) type 1 receptor kinase. Ubiquitin-mediated proteolysis by SMAD-specific E3 ubiquitin ligase SMURF1.
細胞内局在	Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand. Migrates to the nucleus when complexed with SMAD4.

画像



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

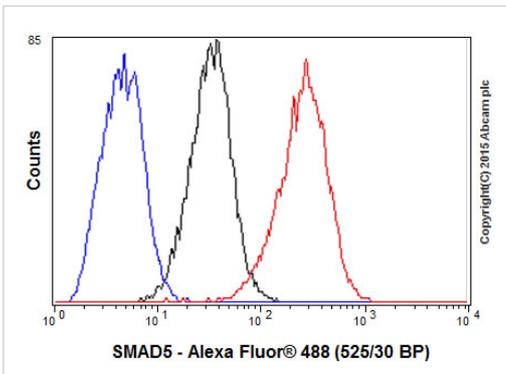
All lanes : Goat Anti-Rabbit IgG H&L (HRP)
(ab97051) at 1/20000 dilution (HRP goat anti-rabbit IgG (H+L))

Predicted band size: 52 kDa

Observed band size: 52 kDa

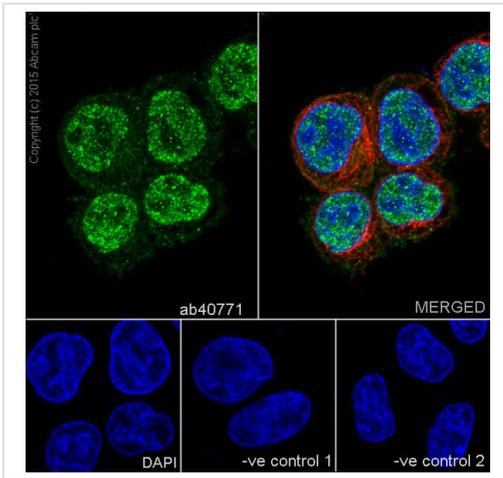
Blocking buffer: 5% NFDm/TBST

Dilution buffer: 5% NFDm/TBST



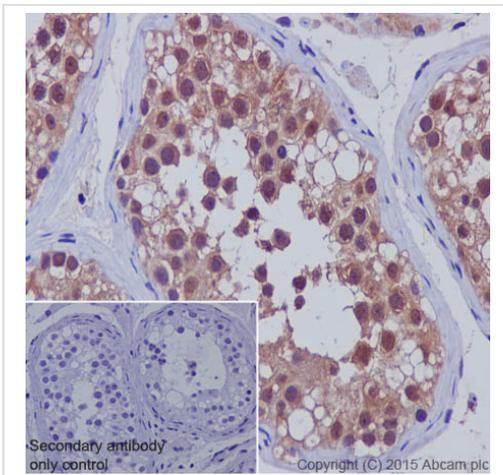
Flow Cytometry - Anti-SMAD5 antibody [EP619Y]
(ab40771)

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 Fluor[®] 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 performed 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.

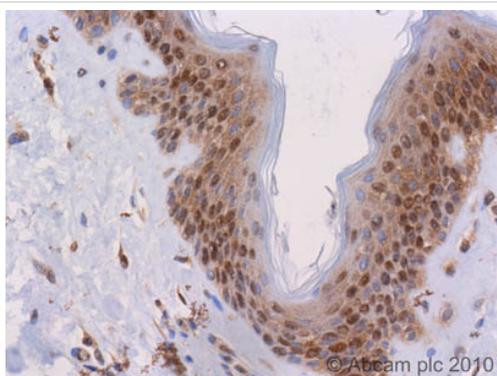


Western blot - Anti-SMAD5 antibody [EP619Y] (ab40771)

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

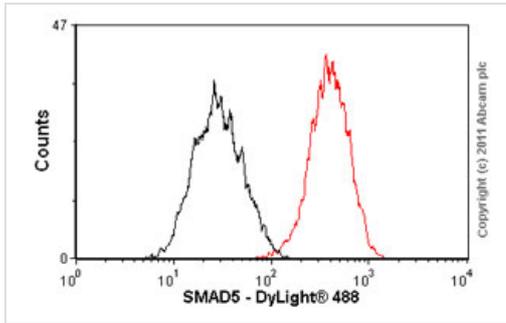
Predicted band size: 52 kDa

Observed band size: 52 kDa



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

Unpurified ab40771 (4 μ g/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 - 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/1x10⁶ 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.

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