abcam

Product datasheet

Anti-SMAD5 antibody [EP619Y] ab40771

リコンビナント RabMAb

4 Abreviews 33 References 画像数 9

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:** Flow Cyt (Intra), IHC-P, WB, ICC/IF, Dot blot
種交差性	**交差種:** Mouse, Rat, Human, African green monkey
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 (intra): PC-12 and HEK293 cells. ICC/IF: HeLa cells. IHC-P: Human testis tissue and human skin tissue.
特記事項	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**.

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

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab40771の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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.

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).

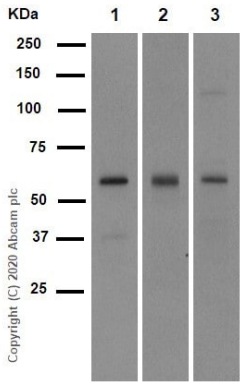
組織特異性
Ubiquitous.

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 1/1000 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

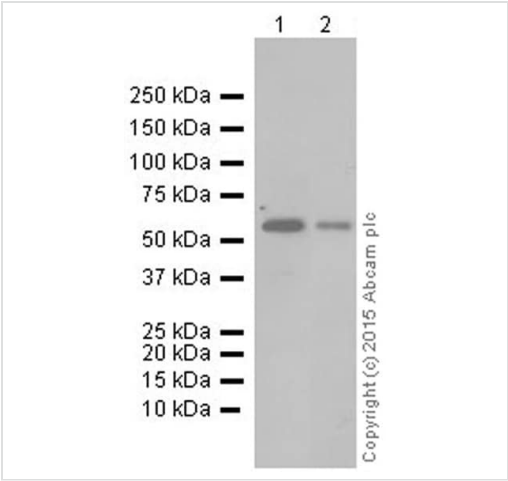
Lysates/proteins at 20 µg per lane.

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)

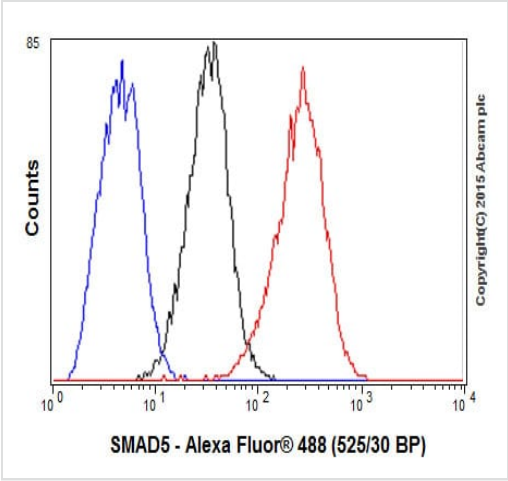
Lane 1 : HEK293 whole cell lysate
Lane 2 : COS-1 whole cell lysate

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))

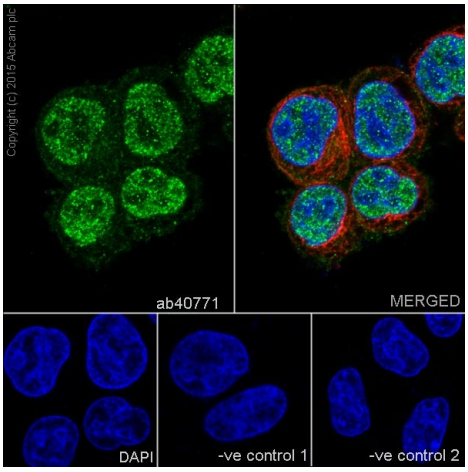
Predicted band size: 52 kDa
Observed band size: 52 kDa

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



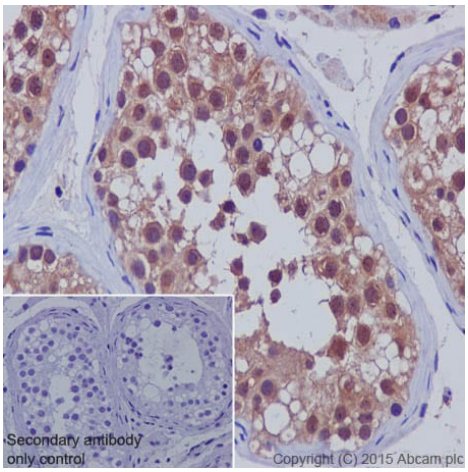
Flow Cytometry (Intracellular) - Anti-SMAD5 antibody [EP619Y] (ab40771)

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).



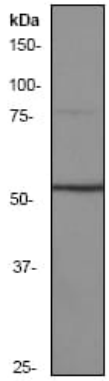
Immunocytochemistry/ Immunofluorescence - Anti-SMAD5 antibody [EP619Y] (ab40771)

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)

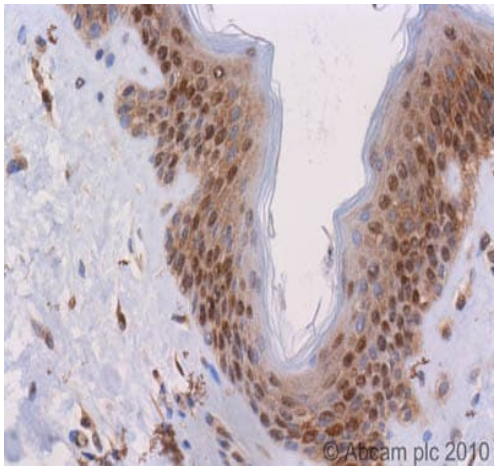
Immunohistochemical staining of paraffin embedded human testis with purified ab40771 at a working dilution of 1/50. The secondary antibody used is **ab97051**, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA 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)

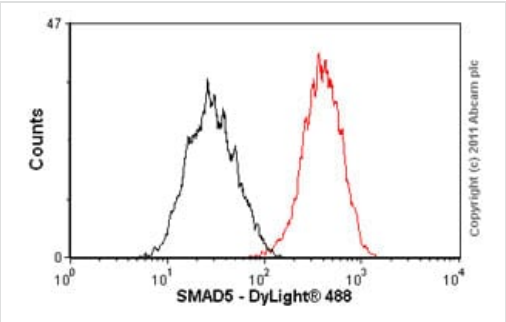
Anti-SMAD5 antibody [EP619Y] (ab40771) at 1/1000 dilution (unpurified) + Cos-1 cell lysate at 10 µg

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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