abcam

Product datasheet

Anti-SMAD5 antibody [EP619Y] - BSA and Azide free ab238952

Recombinant RabMAb

6 Images

Overview

Product name	Anti-SMAD5 antibody [EP619Y] - BSA and Azide free
Description	Rabbit monoclonal [EP619Y] to SMAD5 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Dot blot, WB, IHC-P, ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human, African green monkey
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human testis tissue.
General notes	ab238952 is the carrier-free version of **ab40771**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP619Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab238952 in the following tested applications.

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

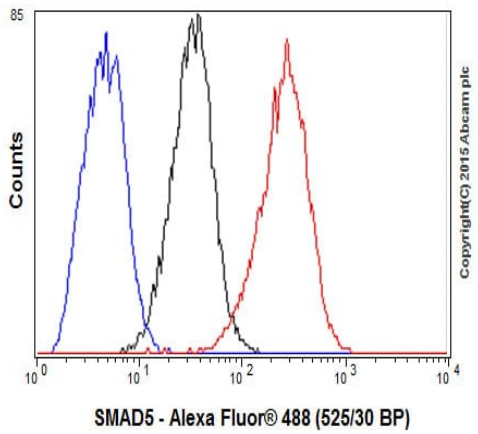
Application	Abreviews	Notes
Dot blot		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 52 kDa (predicted molecular weight: 52 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		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

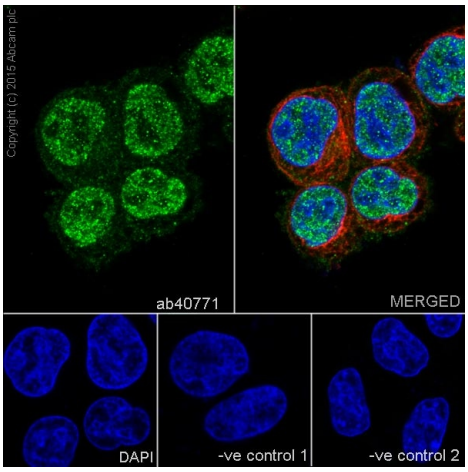
Images



Flow Cytometry (Intracellular) - Anti-SMAD5 antibody [EP619Y] - BSA and Azide free (ab238952)

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).

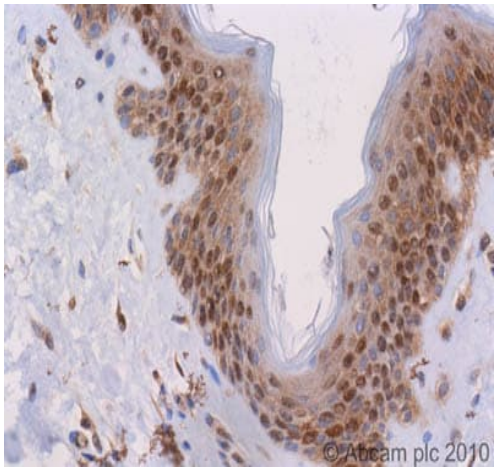
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40771**).



Immunocytochemistry/ Immunofluorescence - Anti-SMAD5 antibody [EP619Y] - BSA and Azide free (ab238952)

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.

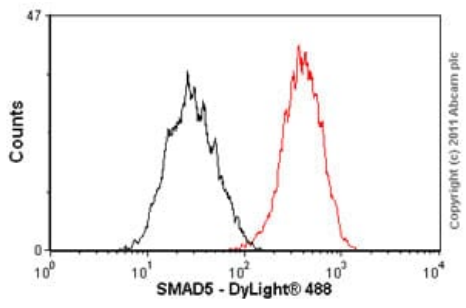
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40771**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD5 antibody [EP619Y] - BSA and Azide free (ab238952)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40771**).

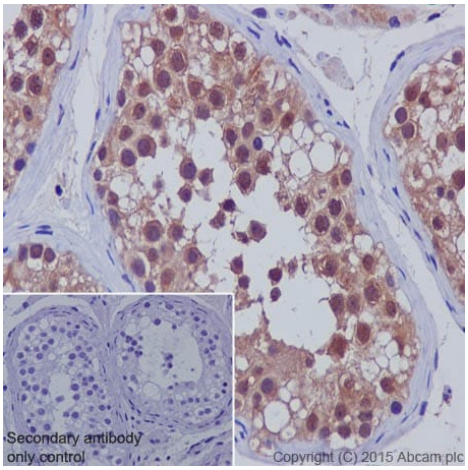


Flow Cytometry (Intracellular) - Anti-SMAD5 antibody [EP619Y] - BSA and Azide free (ab238952)

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.

This data was developed using the same antibody clone in a

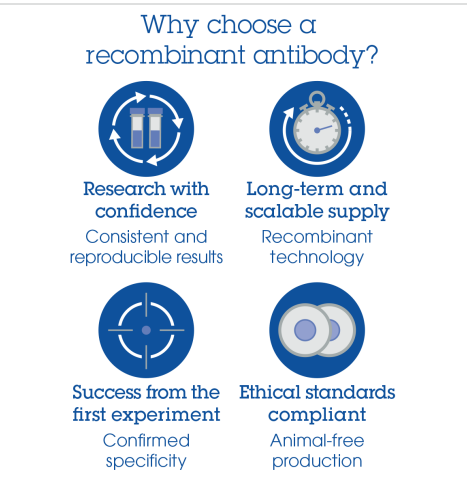
different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40771**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD5 antibody [EP619Y] - BSA and Azide free (ab238952)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40771**).



Anti-SMAD5 antibody [EP619Y] - BSA and Azide free (ab238952)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors