abcam

Product datasheet

Alexa Fluor® 647 Anti-Smad4 antibody [SP306] - C-terminal ab267521

Recombinant

3 Images

Overview

Product name	Alexa Fluor® 647 Anti-Smad4 antibody [SP306] - C-terminal
Description	Alexa Fluor® 647 Rabbit monoclonal [SP306] to Smad4 - C-terminal
Host species	Rabbit
Conjugation	Alexa Fluor® 647. Ex: 652nm, Em: 668nm
Tested applications	**Suitable for:** ICC/IF, IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human normal pancreas tissue. ICC/IF: NIH/3T3 cells.
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**. **This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.** Alexa Fluor® is a registered trademark of Molecular Probes, Inc, a Thermo Fisher Scientific Company. The Alexa Fluor® dye included in this product is provided under an intellectual property license from Life Technologies Corporation. As this product contains the Alexa Fluor® dye, the purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). As this product contains the Alexa Fluor® dye the sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: in manufacturing; (ii) to provide a service, information, or data in return for payment (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are sold for use in research. For information on purchasing a license to this product for purposes other than research, contact

Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008 USA or **outlicensing@thermofisher.com**.

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 long term. Avoid freeze / thaw cycle. Stable for 12 months at -20°C. Store In the Dark.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: 30% Glycerol (glycerin, glycerine), 1% BSA, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	SP306
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab267521 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
ICC/IF		1/100. This product gave a positive signal in NIH/3T3 fixed with 4% formaldehyde (10 mins).
IHC-P		1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Target

Function Common SMAD (co-SMAD) is the coactivator and mediator of signal transduction by TGF-beta (transforming growth factor). Component of the heterotrimeric SMAD2/SMAD3-SMAD4 complex that forms in the nucleus and is required for the TGF-mediated signaling. Promotes binding of the SMAD2/SMAD4/FAST-1 complex to DNA and provides an activation function required for SMAD1 or SMAD2 to stimulate transcription. Component of the multimeric SMAD3/SMAD4/JUN/FOS complex which forms at the AP1 promoter site; required for syngernistic transcriptional activity in response to TGF-beta. May act as a tumor suppressor.

Involvement in disease Defects in SMAD4 are a cause of pancreatic cancer (PNCA) [MIM:260350].
Defects in SMAD4 are a cause of juvenile polyposis syndrome (JPS) [MIM:174900]; also known as juvenile intestinal polyposis (JIP). JPS is an autosomal dominant gastrointestinal hamartomatous polyposis syndrome in which patients are at risk for developing gastrointestinal cancers. The lesions are typified by a smooth histological appearance, predominant stroma, cystic spaces and lack of a smooth muscle core. Multiple juvenile polyps usually occur in a number of Mendelian disorders. Sometimes, these polyps occur without associated features as in JPS;

here, polyps tend to occur in the large bowel and are associated with an increased risk of colon and other gastrointestinal cancers.
Defects in SMAD4 are a cause of juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome (JP/HHT) [MIM:175050]. JP/HHT syndrome phenotype consists of the coexistence of juvenile polyposis (JIP) and hereditary hemorrhagic telangiectasia (HHT) [MIM:187300] in a single individual. JIP and HHT are autosomal dominant disorders with distinct and non-overlapping clinical features. The former, an inherited gastrointestinal malignancy predisposition, is caused by mutations in SMAD4 or BMPR1A, and the latter is a vascular malformation disorder caused by mutations in ENG or ACVRL1. All four genes encode proteins involved in the transforming-growth-factor-signaling pathway. Although there are reports of patients and families with phenotypes of both disorders combined, the genetic etiology of this association is unknown.
Defects in SMAD4 may be a cause of colorectal cancer (CRC) [MIM:114500].

Sequence similarities

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

Domain

The MH1 domain is required for DNA binding.
The MH2 domain is required for both homomeric and heteromeric interactions and for transcriptional regulation. Sufficient for nuclear import.

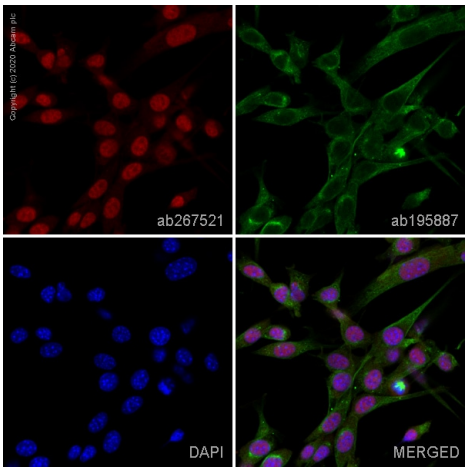
Post-translational modifications

Monoubiquitinated on Lys-519 by E3 ubiquitin-protein ligase TRIM33. Monoubiquitination hampers its ability to form a stable complex with activated SMAD2/3 resulting in inhibition of TGF-beta/BMP signaling cascade. Deubiqitination by USP9X restores its competence to mediate TGF-beta signaling.

Cellular localization

Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand. Migrates to the nucleus when complexed with R-SMAD.

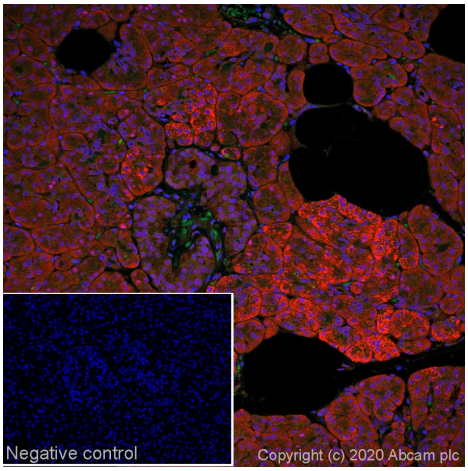
Images



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-Smad4 antibody [SP306] - C-terminal (ab267521)

ab267521 staining Smad4 in NIH3T3 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab267521 at 1/100 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Alexa Fluor® 647 Anti-Smad4 antibody [SP306] - C-terminal (ab267521)

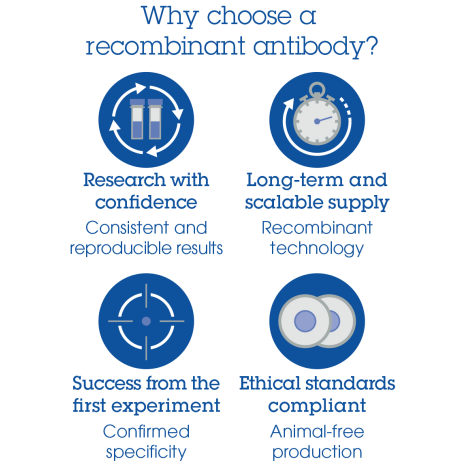
IHC image of Smad4 - C-terminal staining in a section of formalin-fixed paraffin-embedded normal human pancreas*.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) in a Biocare Medical NxGen pressure cooker using retrieval settings of 110°C for 20 minutes. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with ab267521 at 1/100 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.



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