abcam

Product datasheet

PE Anti-Smad3 antibody [EP568Y] ab208751

Recombinant RabMAb

★★★★★ 1 Abreviews 3 Images

Overview

Product name	PE Anti-Smad3 antibody [EP568Y]
Description	PE Rabbit monoclonal [EP568Y] to Smad3
Host species	Rabbit
Conjugation	PE. Ex: 488nm, Em: 575nm
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	Flow Cyt (intra): HepG2 cells ICC/IF: HepG2 cells

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot. Store at +4°C. Do Not Freeze. Store In the Dark.
Storage buffer	pH: 7.4 Preservative: 0.02% Sodium azide Constituents: PBS, 1% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP568Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab208751 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)		1/500.
ICC/IF		1/1000. This product gave a positive signal in HepG2 cells fixed with 4% formaldehyde (10 min)

Target

Function

Receptor-regulated SMAD (R-SMAD) that is an intracellular signal transducer and transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinases. Binds the TRE element in the promoter region of many genes that are regulated by TGF-beta and, on formation of the SMAD3/SMAD4 complex, activates transcription. Also can form a SMAD3/SMAD4/JUN/FOS complex at the AP-1/SMAD site to regulate TGF-beta-mediated transcription. Has an inhibitory effect on wound healing probably by modulating both growth and migration of primary keratinocytes and by altering the TGF-mediated chemotaxis of monocytes. This effect on wound healing appears to be hormone-sensitive. Regulator of chondrogenesis and osteogenesis and inhibits early healing of bone fractures. Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator.

Involvement in disease

Colorectal cancer
Loeys-Dietz syndrome 3

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. Also binds zinc ions which are necessary for the DNA binding.
The MH2 domain is required for both homomeric and heteromeric interactions and for transcriptional regulation. Sufficient for nuclear import.
The linker region is required for the TGFbeta-mediated transcriptional activity and acts synergistically with the MH2 domain.

Post-translational modifications

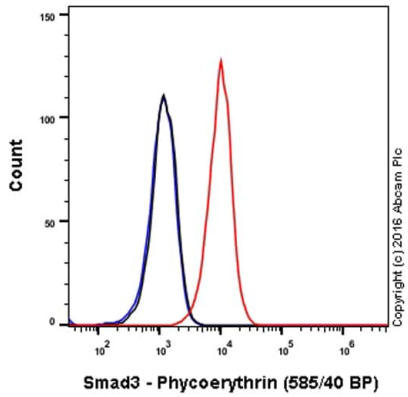
Phosphorylated on serine and threonine residues. Enhanced phosphorylation in the linker region on Thr-179, Ser-204 and Ser-208 on EGF and TGF-beta treatment. Ser-208 is the main site of MAPK-mediated phosphorylation. CDK-mediated phosphorylation occurs in a cell-cycle dependent manner and inhibits both the transcriptional activity and antiproliferative functions of SMAD3. This phosphorylation is inhibited by flavopiridol. Maximum phosphorylation at the G(1)/S junction. Also phosphorylated on serine residues in the C-terminal SXS motif by TGFBR1 and ACVR1. TGFBR1-mediated phosphorylation at these C-terminal sites is required for interaction with SMAD4, nuclear location and transactivational activity, and appears to be a prerequisite for the TGF-beta mediated phosphorylation in the linker region. Dephosphorylated in the C-terminal SXS motif by PPM1A. This dephosphorylation disrupts the interaction with SMAD4, promotes nuclear export and terminates TGF-beta-mediated signaling. Phosphorylation at Ser-418 by CSNK1G2/CK1 promotes ligand-dependent ubiquitination and subsequent proteasome degradation, thus inhibiting SMAD3-mediated TGF-beta responses. Phosphorylated by PDPK1. Acetylation in the nucleus by EP300 in the MH2 domain regulates positively its transcriptional activity and is enhanced by TGF-beta.
Ubiquitinated. Monoubiquitinated, leading to prevent DNA-binding. Deubiquitination by USP15

alleviates inhibition and promotes activation of TGF-beta target genes.
Poly-ADP-ribosylated by PARP1 and PARP2. ADP-ribosylation negatively regulates SMAD3 transcriptional responses during the course of TGF-beta signaling.

Cellular localization

Cytoplasm. Nucleus. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4 (PubMed:15799969). Through the action of the phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1 (PubMed:16751101, PubMed:19289081). Co-localizes with LEMD3 at the nucleus inner membrane (PubMed:15601644). MAPK-mediated phosphorylation appears to have no effect on nuclear import (PubMed:19218245). PDPK1 prevents its nuclear translocation in response to TGF-beta (PubMed:17327236).

Images

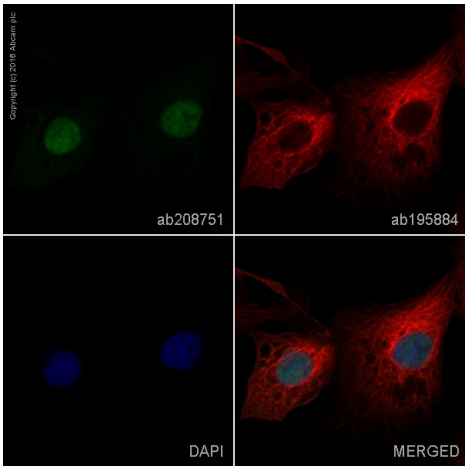


Flow Cytometry (Intracellular) - PE Anti-Smad3 antibody [EP568Y] (ab208751)

Overlay histogram showing HepG2 cells stained with ab208751 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 90% methanol (-20°C) for 30 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab208751, 1/500 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was rabbit IgG (monoclonal) Phycoerythrin (**ab209478**) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20 mW Solid State Blue Laser (488nm) and 585/40 bandpass filter



Immunocytochemistry/ Immunofluorescence - PE Anti-Smad3 antibody [EP568Y] (ab208751)

ab208751 staining Smad3 in HepG2 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 ab208751 at 1/1000 dilution (**pseudocolored in green**) and **ab195884**, Rat monoclonal to Tubulin (Alexa Fluor® 647), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

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

PE Anti-Smad3 antibody [EP568Y] (ab208751)

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