

Product datasheet

Alexa Fluor® 647 Anti-Smad3 antibody [EP568Y] ab204461

Recombinant RabMAb

2 Images

Overview

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| Product name | Alexa Fluor® 647 Anti-Smad3 antibody [EP568Y] |
| Description | Alexa Fluor® 647 Rabbit monoclonal [EP568Y] to Smad3 |
| Host species | Rabbit |
| Conjugation | Alexa Fluor® 647. Ex: 652nm, Em: 668nm |
| Tested applications | Suitable for: ICC/IF |
| Species reactivity | Reacts with: Human Predicted to work with: Mouse, Rat  |
| Immunogen | Synthetic peptide within Human Smad3 aa 200-300 (internal sequence). The exact sequence is proprietary. Database link: P84022 (Peptide available as ab173094) |
| Positive control | ICC/IF: HepG2 cells. |
| General notes | This product is a recombinant monoclonal antibody, which offers several advantages including: <ul style="list-style-type: none"> - 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 . 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 |

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Properties

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| Form | Liquid |
| Storage instructions | Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle. Store In the Dark. |
| Storage buffer | pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 1% BSA, 30% Glycerol (glycerin, glycerine) |
| Purity | Protein A purified |
| Clonality | Monoclonal |
| Clone number | EP568Y |
| Isotype | IgG |

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab204461 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. |

Target

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| 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.

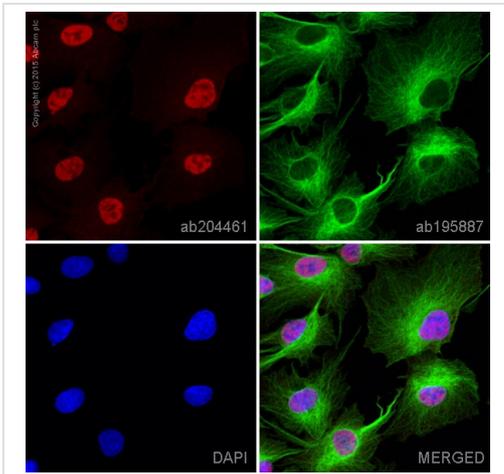
Ubiquitinated. Monoubiquitinated, leading to prevent DNA-binding. Deubiquitination by USP15 alleviates inhibition and promotes activation of TGF-beta target genes.

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



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-Smad3 antibody [EP568Y] (ab204461)

ab204461 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 ab204461 at 1/100 dilution (shown in red) and ab195887, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

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

This product also gave a positive signal under the same testing conditions in HepG2 cells fixed with 100% methanol (5 min).

Why choose a recombinant antibody?

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|  Research with confidence Consistent and reproducible results |  Long-term and scalable supply Recombinant technology |
|  Success from the first experiment Confirmed specificity |  Ethical standards compliant Animal-free production |

Alexa Fluor® 647 Anti-Smad3 antibody [EP568Y] (ab204461)

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

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