abcam

Product datasheet

Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free ab172202

Recombinant RabMAb

11 Images

Overview

Product name	Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free
Description	Rabbit monoclonal [EP823Y] to Smad3 (phospho S423 + S425) - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF, Dot blot
Species reactivity	**Reacts with:** Mouse, Human, Drosophila melanogaster
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
General notes	ab172202 is the carrier-free version of ab52903. 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	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP823Y
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab172202 in the following tested applications.

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

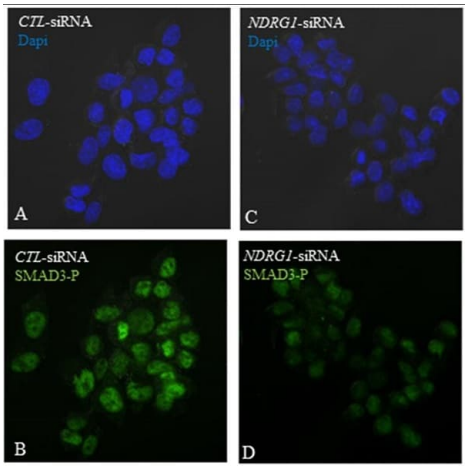
Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Predicted molecular weight: 48 kDa. Avoid using milk, casein, and phopshorylated proteins in general in the blocking buffer and in the antibody diluent. We recommend a solution of 5% BSA (bovine serum albumin).
ICC/IF		Use at an assay dependent concentration.
Dot blot		Use at an assay dependent concentration.

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

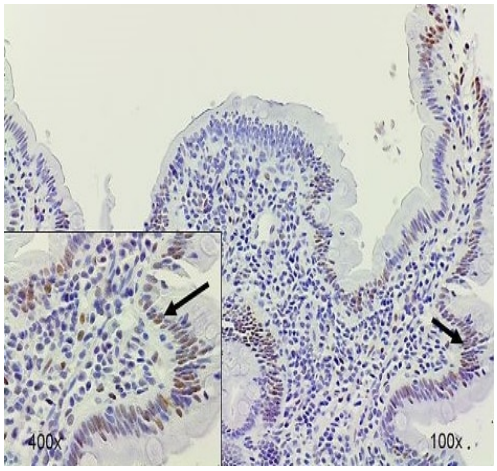


Immunocytochemistry/ Immunofluorescence - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free (ab172202)

Image from Tang MK et al. PLoS One. 2013;8(3):e59477. Fig 12.; doi: 10.1371/journal.pone.0059477.

TGF-β1 signaling is impaired in *NDRG1*-silenced MEFs. $PML^{+/+}$ mouse embryonic fibroblasts (MEFs) were transfected with either *CTL*-siRNAs (A & B) or *NDRG1*-siRNAs (C & D) and induced with100 ng/ml TGF-β1. Immunofluorescent staining revealed intense nuclear staining for phosphorylated SMAD3 (SMAD3-P, ab52903) in *CTL*-siRNA treated MEFs (B) while only weak nuclear staining for MEFs treated with *NDRG1*-siRNA (D).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52903).

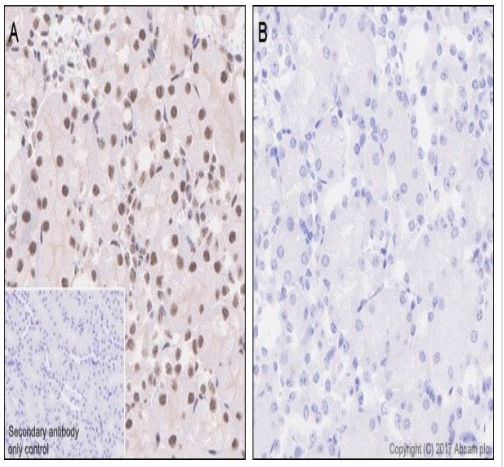


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free (ab172202)

Image from Syed S et al. PLoS Negl Trop Dis. 2018;12(2):e0006224. Fig 4.; doi: 10.1371/journal.pntd.0006224.

Representative IHC photomicrographs from an Environmental enteropathy (EE) duodenal biopsy showing p-SMAD3 staining (ab52903) in only the epithelium (arrows).

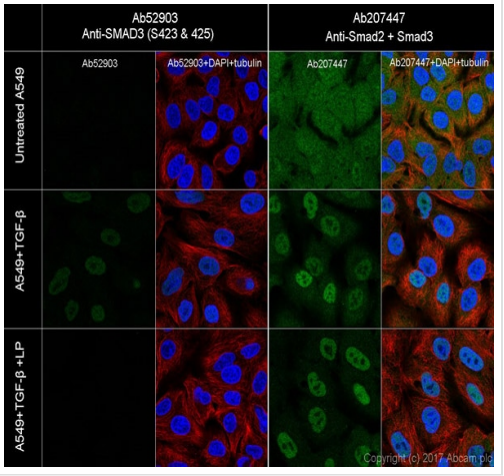
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52903).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free (ab172202)

Purified ab52903 staining Smad3 in Mouse kidney tissue sections by Immunohistochemistry (Formalin/PFA fixed paraffin embedded sections). Tissue was fixed with paraffin and antigen retrieval was by heat mediation using ab93684 (Tris/EDTA buffer, pH 9.0). Samples were incubated with primary antibody at a 1/200 dilution. A ready to use rabbit specific IHC polymer detection kit HRP/DAP (ab209101). Hematoxylin was used as a counterstain. Nuclear and weakly cytoplasmic staining on mouse kidney without alkaline phosphatase treatment (image A). No signal can be detected when tissues were treated with alkaline phosphatase (image B).

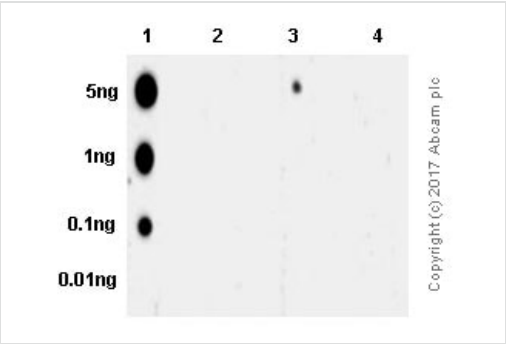
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52903).



Immunocytochemistry/ Immunofluorescence - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free (ab172202)

Immunocytochemistry/Immunofluorescence analysis of A549 +/- TGFβ (5ng/ml, 24h) and A549 + TGFβ (5ng/ml, 24h) + Lamda phosphatase (LP) cells. Smad3 (phospho S423 + S425) was labelled with purified ab52903 at a dilution of 1/100 dilution, while Smad3 was labelled with ab207447 at a dilution of 1/500 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% triton X-100. ab150077 (goat anti-rabbit IgG Alexa Fluor® 488) (1/1000) was used as the secondary antibody. The cells were co-stained with ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) 1/200. Nuclei counterstained with DAPI (blue). Control: PBS instead of the primary antibody.

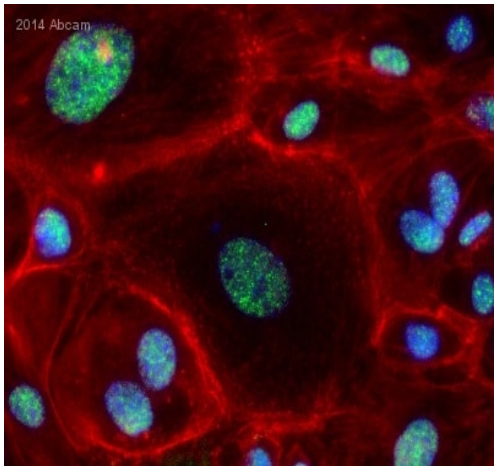
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52903).



Dot Blot - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free (ab172202)

Dot blot analysis of Smad 3 (phospho S423 + S425) phospho peptide (Lane 1), Smad 3 (phospho S423) phospho peptide (Lane 2), Smad 3 (phospho S425) phospho peptide (Lane 3) and Smad 3 non-phospho peptide (Lane 4) labelling Smad 3 (phospho S423 + S425) with ab52903 at a dilution of 1/1000. A Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) was used as the secondary antibody at a dilution of 1/20,000. Blocking and dilution buffer: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52903).

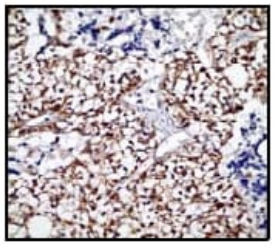


Immunocytochemistry/ Immunofluorescence - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free (ab172202)

This image is courtesy of an anonymous Abreview.

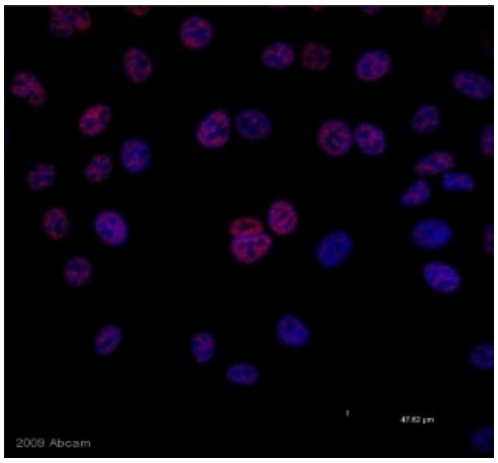
ab52903 staining Smad3 in mouse primary embryonic epicardial cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% formaldehyde, permeabilized with 0.5% Triton X-100 and blocked with PBS + 1% BSA + 10% goat serum + 0.1% Triton X-100 for 1 hour at 20°C. Samples were incubated with primary antibody (1/100 in PBS + 1% BSA + 10% goat serum + 0.1% Triton X-100) for 16 hours at 4°C. An Alexa Fluor®488-conjugated goat anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52903).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free (ab172202)

Immunohistochemical analysis of Smad3 in paraffin embedded human liver carcinoma tissue using ab52903 at 1/100 dilution.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52903).

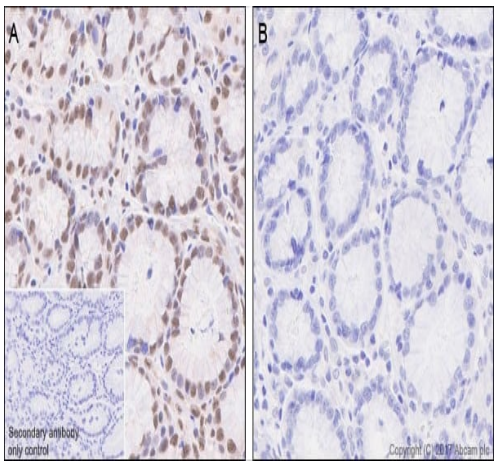


Immunocytochemistry/ Immunofluorescence - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free (ab172202)

This image is courtesy of an Abreview submitted by Aaron Gardner.

ab52903 staining Smad3 (phospho S423 + S425) in human TII Pneumocyte A549 cells by Immunocytochemistry/ Immunofluorescence. Cells were fixed with paraformaldehyde and permeabilized with 0.1% Triton x100 before blocking with 3% BSA for 1 hour at RT. Samples were incubated with primary antibody (1/200: in 3% BSA in 1x PBST) for 24 hours at 4°C. A TRITC-conjugated goat polyclonal to rabbit IgG was used as secondary antibody at 1/200 dilution.

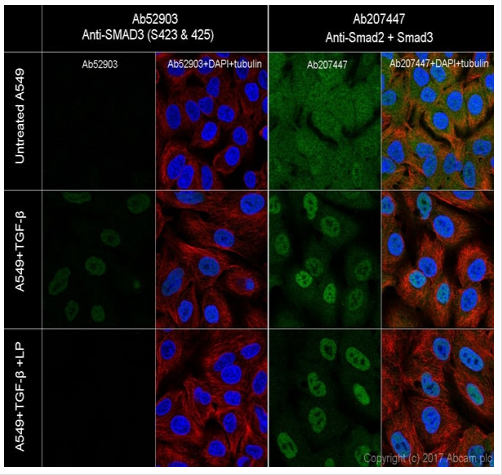
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52903).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free (ab172202)

This IHC data was generated using the same anti-phospho Smad3 S423/425 antibody clone, EP823Y, in a different buffer formulation (cat# ab52903).

Ab52903 staining Smad3 in Human stomach tissue sections by Immunohistochemistry (Formalin/PFA fixed paraffin embedded sections). Tissue was fixed with paraffin and antigen retrieval was by heat mediation using ab93684 (Tris/EDTA buffer, Ph9.0). Samples were incubated with primary antibody at a 1/200 dilution. A ready to use rabbit specific IHC polymer detection kit HRP/DAP (ab209101). Hematoxylin was used as a counterstain. Nuclear and weakly cytoplasmic staining on human stomach without alkaline phosphatase treatment (image A). No signal can be detected when tissues were treated with alkaline phosphatase (image B).



Immunocytochemistry/ Immunofluorescence - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free (ab172202)

This ICC/IF data was generated using the same anti-phospho Smad3 S423/425 antibody clone, EP823Y, in a different buffer formulation (cat# ab52903).

Immunocytochemistry/Immunofluorescence analysis of A549 +/- TGFβ (5ng/ml, 24h) and A549 + TGFβ (5ng/ml, 24h) + Lamda phosphatase (LP) cells. Smad3 (phospho S423 + S425) was labelled with ab52903 at a dilution of 1/100 dilution, while Smad3 was labelled with ab207447 at a dilution of 1/500 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% triton X-100. ab150077 (goat anti-rabbit IgG Alexa Fluor® 488) (1/1000) was used as the secondary antibody. The cells were co-stained with ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) 1/200. Nuclei counterstained with DAPI (blue). Control: PBS instead of the primary antibody.

Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] - BSA and Azide free (ab172202)

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