abcam

Product datasheet

Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] ab52903

Recombinant RabMAb

20 Abreviews 507 References 18 Images

Overview

Product name	Anti-Smad3 (phospho S423 + S425) antibody [EP823Y]
Description	Rabbit monoclonal [EP823Y] to Smad3 (phospho S423 + S425)
Host species	Rabbit
Specificity	This antibody detects Smad3 phosphorylated on Serine 423 and Serine 425. This Smad3 antibody may also detect Smad1, Smad2 and Smad5 phosphorylated at the equivalent sites.
Tested applications	**Suitable for:** WB, ICC/IF, ChIC/CUT&RUN-seq, IHC-P, Dot blot **Unsuitable for:** Flow Cyt or IP
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Drosophila melanogaster
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HL-60 treated with TGF-ß cell lysates; A549 untreated and treated with 5ng/ml TGF-ß1 for 24 hours whole cell lysates; F9 whole cell lysate. IHC-P: Human stomach and liver carcinoma tissue; Mouse kidney tissue; Environmental enteropathy (EE) duodenal biopsy. ICC/IF: TGFß treated A549 cells; PML+/+ mouse embryonic fibroblasts (MEFs) were transfected with either CTL-siRNAs or NDRG1-siRNAs; Mouse primary embryonic epicardial 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**. Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP823Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab52903 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
WB	★★★★☆ (13)	1/2000. Predicted molecular weight: 48 kDa.
ICC/IF	★★★★★ (3)	1/100 - 1/250.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
IHC-P	★★★★★ (3)	1/100 - 1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. The secondary antibody is rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).
Dot blot		1/1000.

Application notes Is unsuitable for Flow Cyt or IP.

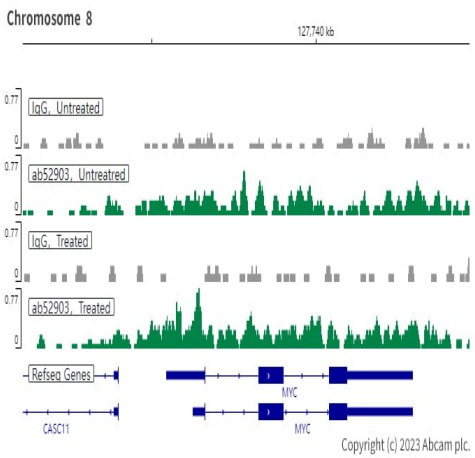
Target

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

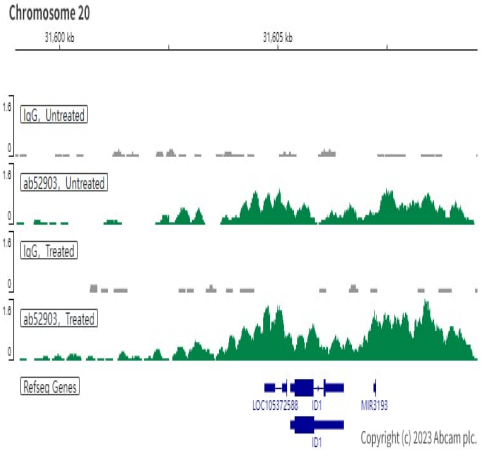
Sequence similarities	Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.
Domain	The MH1 domain is required for DNA binding. Also binds zinc ions which are necessary for the DNA binding. The MH2 domain is required for both homomeric and 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



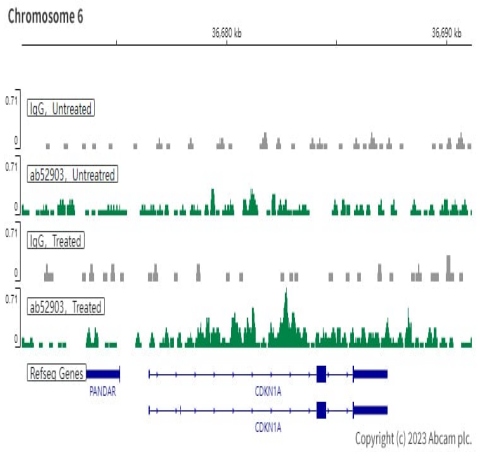
ChIC/CUT&RUN sequencing - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/µL, 2.5 x 10^5 A549 (Human lung carcinoma cell line) cells treated with hTGF-β1 (7 ng/mL 1 h) and 5 µg of ab52903 [EP823Y]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



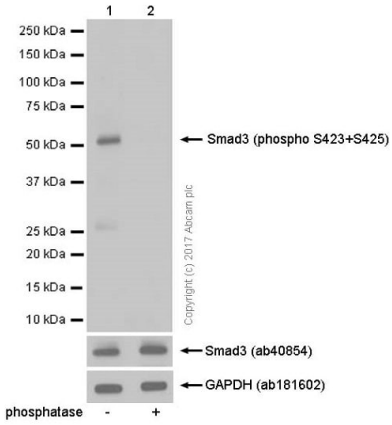
ChIC/CUT&RUN sequencing - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/µL, 2.5 x 10^5 A549 (Human lung carcinoma cell line) cells treated with hTGF-β1 (7 ng/mL 1 h) and 5 µg of ab52903 [EP823Y]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



ChIC/CUT&RUN sequencing - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/µL, 2.5 x 10^5 A549 (Human lung carcinoma cell line) cells treated with hTGF-β1 (7 ng/mL 1 h) and 5 µg of ab52903 [EP823Y]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

All lanes : Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903) at 1/2000 dilution (purified)

Lane 1 : F9 (Mouse embryonic testicular cancer epithelial cell) whole cell lysates
Lane 2 : F9 (Mouse embryonic testicular cancer epithelial cell) whole cell lysates. Then the membrane was incubated with phosphatase.

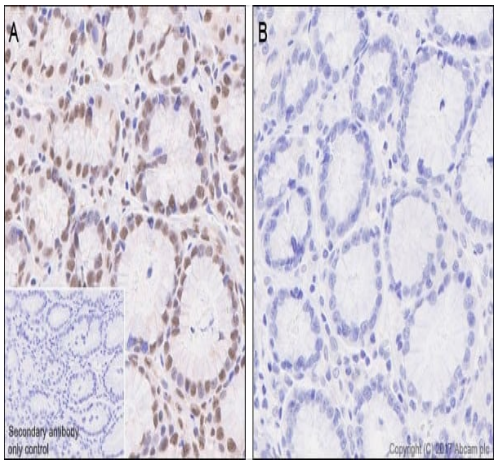
Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 48 kDa
Observed band size: 50 kDa

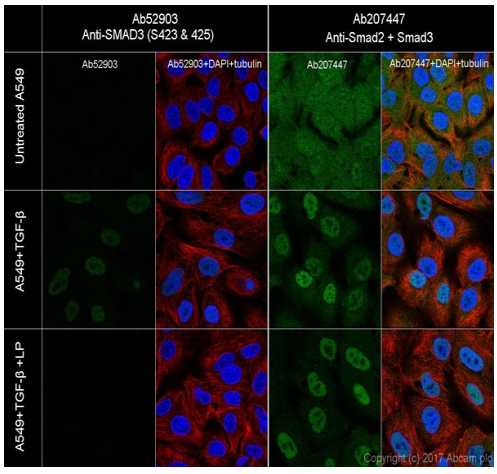
Exposure time: 1 minute

Blocking and diluting buffer: 5% NFDM/TBST.



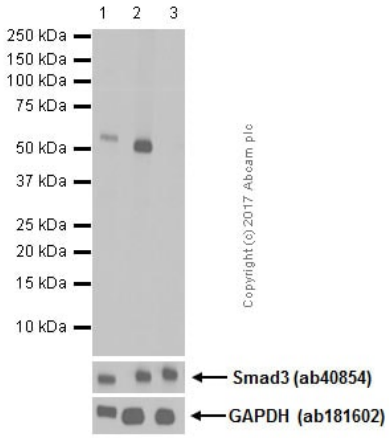
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

Purified 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] (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 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.



Western blot - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

All lanes : Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903) at 1/1000 dilution (purified)

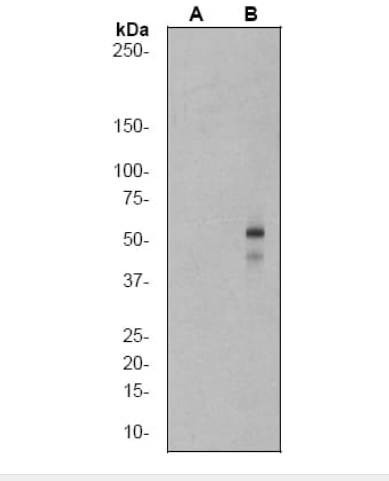
Lane 1 : A549 whole cell lysate
Lane 2 : A549 treated with 5ng/ml TGF-ß1 for 24 hours whole cell lysate
Lane 3 : A549 treated with 5ng/ml TGF-ß1 for 24 hours whole cell lysate, the membrane was incubated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 48 kDa
Observed band size: 55 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

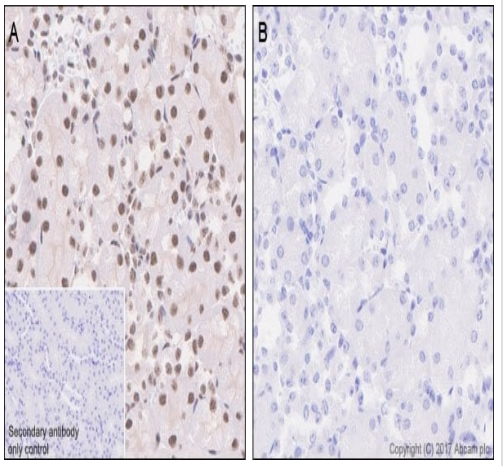


Western blot - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

All lanes : Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903) at 1/2000 dilution

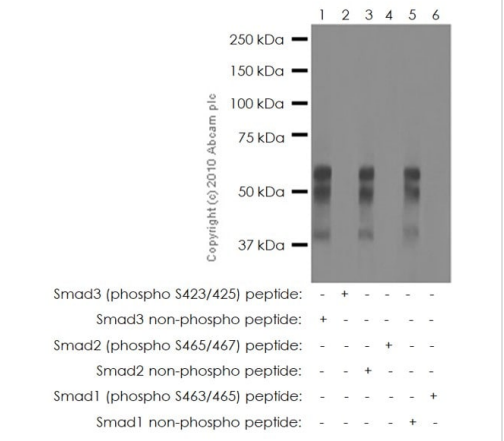
Lane 1 : (A) HL-60 cell lysates at 10µg untreated
Lane 2 : (B) HL-60 cell lysates at 10µg treated with TGF.

Predicted band size: 48 kDa
Observed band size: 55 kDa
Additional bands at: 45 kDa. We are unsure as to the identity of these extra bands.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

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).



Western blot - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

All lanes : Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903) at 1/1000 dilution

Lane 1 : HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad3 non-phospho peptide
Lane 2 : HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad3 (phospho S423/425) peptide
Lane 3 : HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad2 non-phospho peptide
Lane 4 : HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad2 (phospho S465/467) peptide
Lane 5 : HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad1 non-phospho peptide
Lane 6 : HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad1 (phospho S463/465) peptide

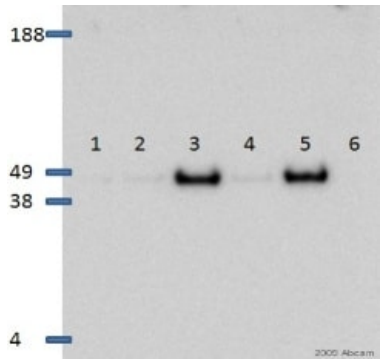
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/2000 dilution

Predicted band size: 48 kDa
Observed band size: 55 kDa

Exposure time: 3 minutes

Blocking and diluting buffer and concentration: 5% NFDM/TBST.



Western blot - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

This image is a courtesy of Aaron Gardner

All lanes : Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903) at 1/1000 dilution

Lane 1 : Lysate prepared from untreated human A549 cells
Lane 2 : Lysate prepared from untreated human A549 cells for 30min
Lane 3 : Lysate prepared from TGF-ß1 cells at 10ng/ml for 30min
Lane 4 : Lysate prepared from TNF-a cells at 20ng/ml for 30min
Lane 5 : Lysate prepared from TGF-ß1 and TNF-a cells at above doses for 30min
Lane 6 : Blank DMEM media

Lysates/proteins at 20 µg per lane.

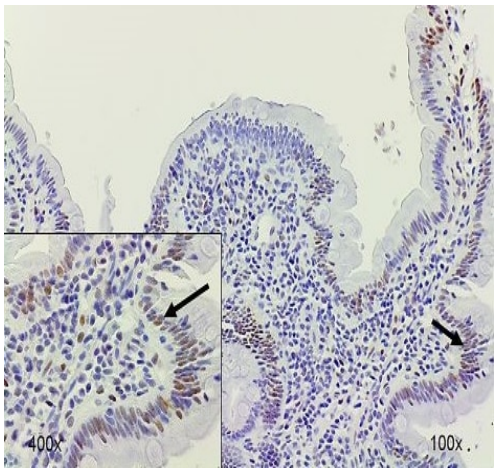
Secondary
All lanes : Donkey Anti-Rabbit IgG H&L (HRP) (**ab16284**)

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 48 kDa
Observed band size: 48 kDa

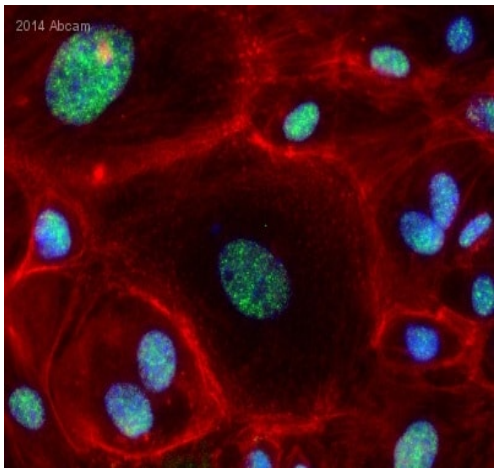
Exposure time: 1 hour



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

Image from Syed S et al., PLoS Negl Trop Dis. 2018;12(2):e0006224. Fig 4.; doi: 10.1371/journal.pntd.0006224. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

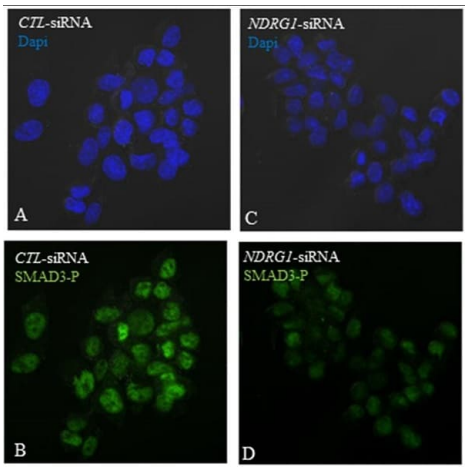
Representative IHC photomicrographs from an Environmental enteropathy (EE) duodenal biopsy showing p-SMAD3 staining (ab52903) in only the epithelium (arrows).



Immunocytochemistry/ Immunofluorescence - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

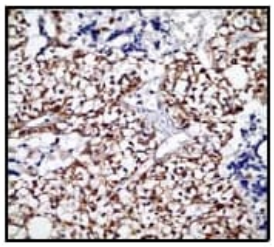
This image is courtesy of an anonymous Abreview

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.



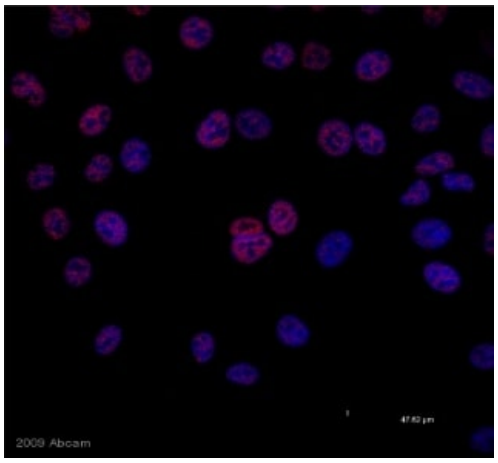
Immunocytochemistry/ Immunofluorescence - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

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).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

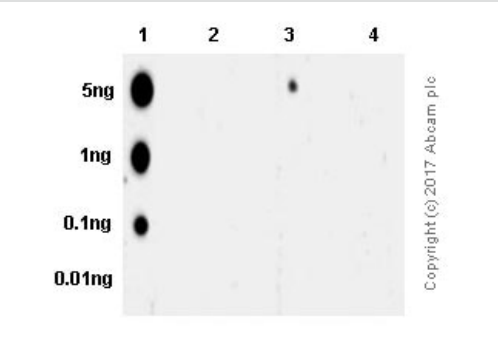
Immunohistochemical analysis of Smad3 in paraffin embedded human liver carcinoma tissue using ab52903 at 1/100 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

This image is a courtesy of 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.



Dot Blot - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

Dot blot analysis of human 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.

Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903)

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