abcam

Product datasheet

Anti-Smad3 antibody [EP568Y] - BSA and Azide free ab157372

KO VALIDATED Recombinant RabMAb

21 Images

Overview

Product name	Anti-Smad3 antibody [EP568Y] - BSA and Azide free
Description	Rabbit monoclonal [EP568Y] to Smad3 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ChIC/CUT&RUN-seq, IHC-P, Sandwich ELISA, WB, ICC/IF, Flow Cyt (Intra) **Unsuitable for:** IP
Species reactivity	**Reacts with:** Rat, Human **Predicted to work with:** Mouse
Immunogen	Synthetic peptide corresponding to Human Smad3 (internal sequence).
Positive control	WB: A549 and HeLa cell lysate; Human kidney tissue lysate. ICC/IF: HepG2 and human granulosa cells. IHC-P: Human breast carcinoma, gastric adenocarcinoma, lung adenocarcinoma, colonic adenocarcinoma, glioma and liver tissues. Flow Cyt (intra): HT-29 and HCT116 cells.
General notes	ab157372 is the carrier-free version of **ab40854**. 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
Purification notes	Protein-A purification via MabSelect SuRe
Clonality	Monoclonal
Clone number	EP568Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab157372 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
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
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.
Sandwich ELISA		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 55 kDa (predicted molecular weight: 48 kDa).
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

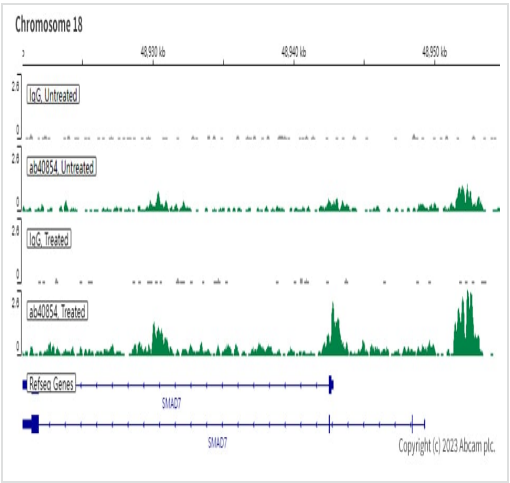
Application notes Is unsuitable for IP.

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,

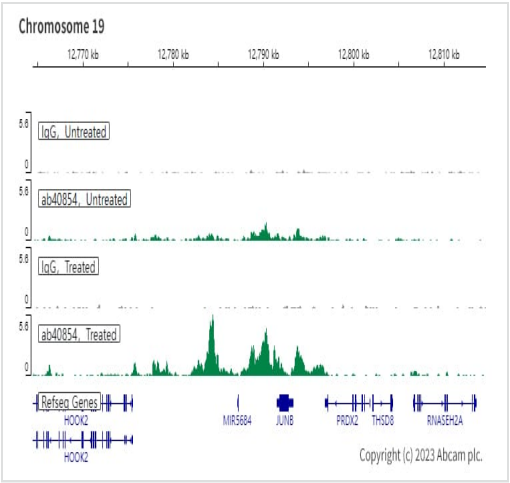
	on formation of the SMAD3/SMAD4 complex, activates transcription. Also can form a SMAD3/SMAD4/JUN/FOS complex at the AP-1/SMAD site to regulate TGF-beta-mediated transcription. Has an inhibitory effect on wound healing probably by modulating both growth and migration of primary keratinocytes and by altering 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 antibody [EP568Y] - BSA and Azide free (ab157372)

This data was developed using the same antibody clone in a different buffer formulation (**ab40854**).
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 **ab40854** [EP568Y]. 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.



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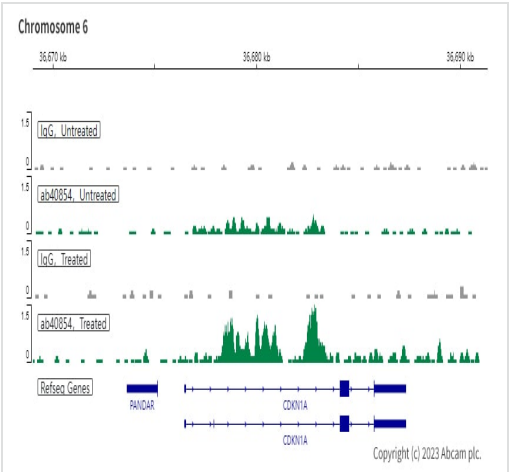

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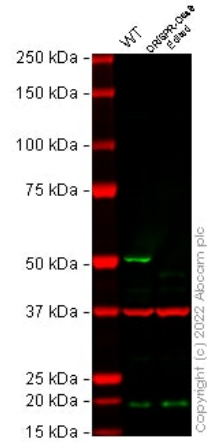


Western blot - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

All lanes : Anti-Smad3 antibody [EP568Y] (**ab40854**) at 1/1000 dilution

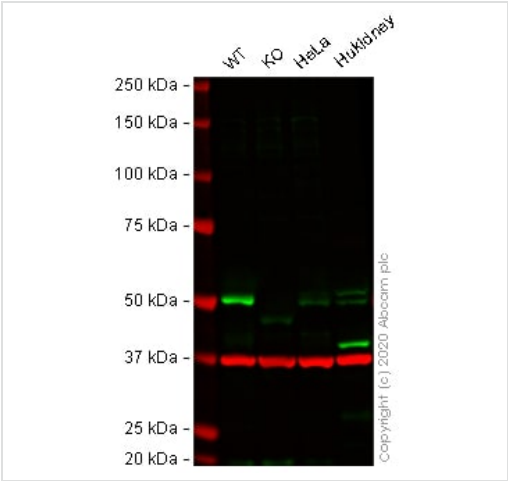
Lane 1 : Wild-type A549 cell lysate
Lane 2 : SMAD3 CRISPR-Cas9 edited A549 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

False colour image of Western blot: Anti-Smad3 antibody [EP568Y] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab40854** was shown to bind specifically to Smad3. A band was observed at 50 kDa in wild-type A549 cell lysates with no signal observed at this size in SMAD3 CRISPR-Cas9 edited cell line **ab277888** (CRISPR-Cas9 edited cell lysate None). The band observed in the CRISPR-Cas9 edited lysate lane below 50 kDa is likely to represent a truncated form of Smad3. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and SMAD3 CRISPR-Cas9 edited A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

All lanes : Anti-Smad3 antibody [EP568Y] (**ab40854**) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : SMAD3 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : Human Kidney cell lysate

Lysates/proteins at 20 µg per lane.

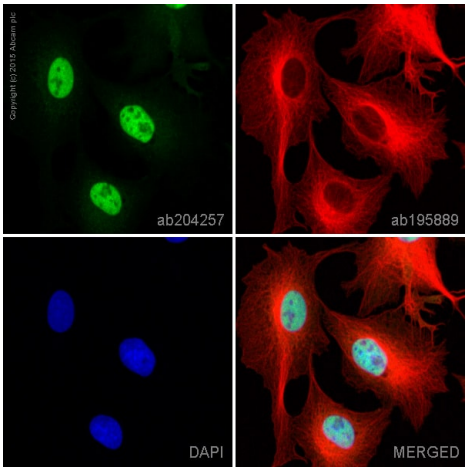
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab40854**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab40854** observed at 48 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab40854 was shown to react with Smad3 in western blot. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab40854** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



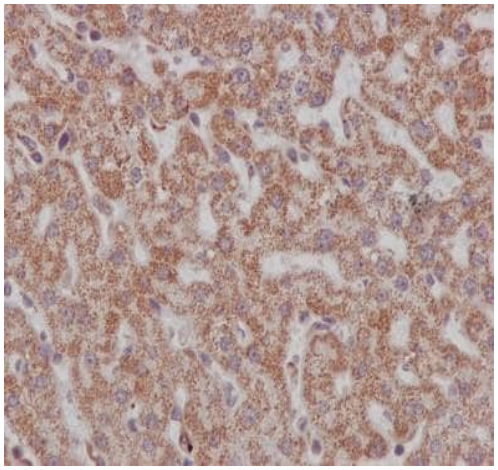
Immunocytochemistry/ Immunofluorescence - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Clone EP568Y (ab157372) has been successfully conjugated by Abcam. This image was generated using Anti-Smad3 antibody [EP568Y] (Alexa Fluor® 488). Please refer to **ab204257** for protocol details.

ab204257 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 **ab204257** at 1/100 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), 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).

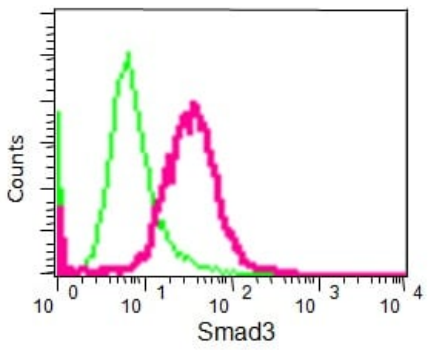
This product also gave a positive signal under the same testing conditions in HepG2 cells fixed with 100% methanol (5 min).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling Smad3 with purified **ab40854** at 1/2000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Counterstained with Hematoxylin.

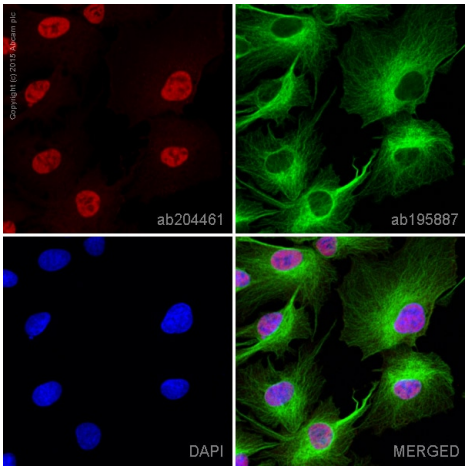
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40854**).



Flow Cytometry (Intracellular) - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Intracellular Flow Cytometry analysis of HT-29 cells labelling Smad3 with purified **ab40854** at 1/210 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Green - Isotype control, rabbit monoclonal IgG.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40854**).



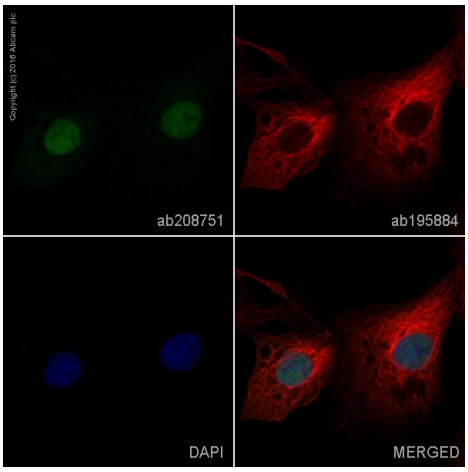
Immunocytochemistry/ Immunofluorescence - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Clone EP568Y (ab157372) has been successfully conjugated by Abcam. This image was generated using Anti-Smad3 antibody [EP568Y] (Alexa Fluor® 647). Please refer to **ab204461** for protocol details.

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).

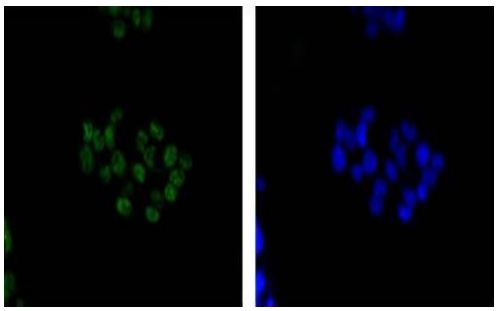


Immunocytochemistry/ Immunofluorescence - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Clone EP568Y (ab157372) has been successfully conjugated by Abcam. This image was generated using Anti-Smad3 antibody [EP568Y] (PE). Please refer to **ab208751** for protocol details.

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).

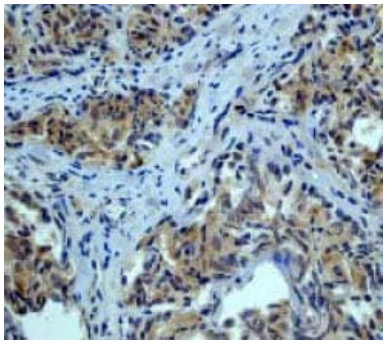
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Immunocytochemsitry/Immunofluorescence analysis of HepG2 cells labelling Smad3 (green) with purified **ab40854** at 1/2000. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/200) was used as the secondary antibody. Counterstained with DAPI (blue).

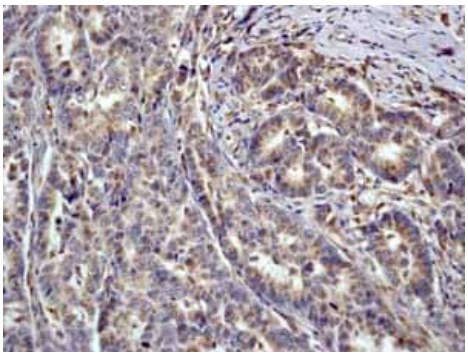
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40854**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate carcinoma tissue labelling unpurified **ab40854** at 1/100 dilution.

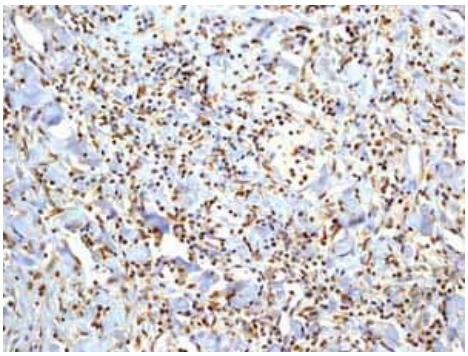
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40854**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling unpurified **ab40854**.

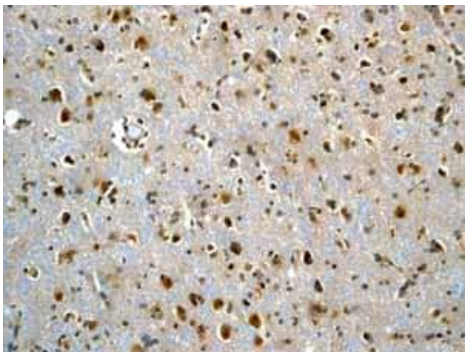
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40854**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gastric adenocarcinoma tissue labelling unpurified **ab40854**.

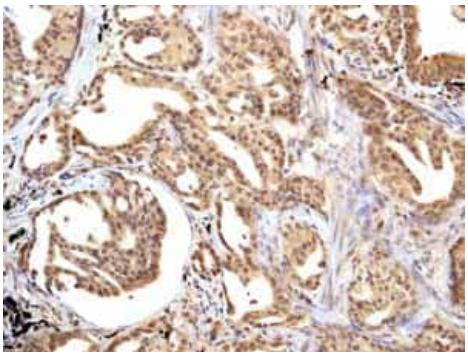
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40854**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human glioma tissue labelling unpurified **ab40854**.

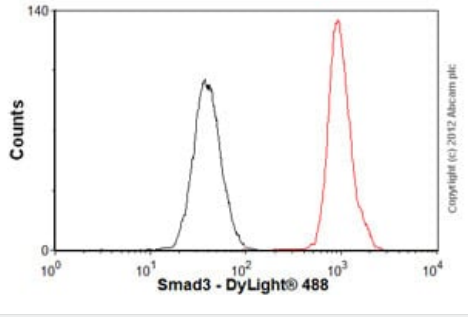
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40854**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung adenocarcinoma tissue labelling unpurified **ab40854**.

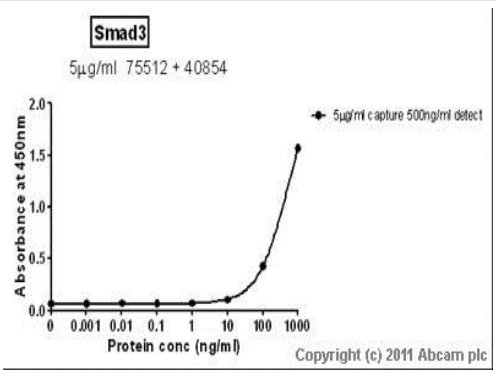
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40854**).



Flow Cytometry (Intracellular) - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

Overlay histogram showing HCT116 cells stained with unpurified **ab40854** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (**ab40854**, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

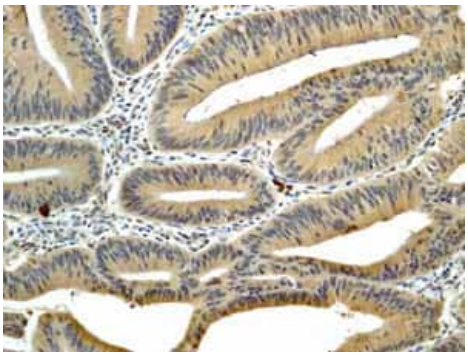
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40854**).



Sandwich ELISA - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

This ELISA data was generated using the same anti-Smad3 antibody clone, EP568Y, in a different buffer formulation (cat# **ab40854**).

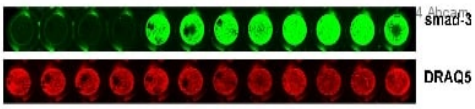
Standard Curve for Smad3 (Analyte: **Smad3 protein (His tag) (ab89353, unpurified)**); dilution range 1pg/ml to 1µg/ml using Capture Antibody **Mouse monoclonal [AF9F7] to Smad3 (ab75512)** at 5µg/ml and Detector Antibody **Rabbit monoclonal [EP568Y] to Smad3 (ab40854)** at 0.5µg/ml.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

This IHC data was generated using the same anti-Smad3 antibody clone, EP568Y, in a different buffer formulation (cat# **ab40854**).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colonic adenocarcinoma tissue labelling Smad3 with unpurified **ab40854**.

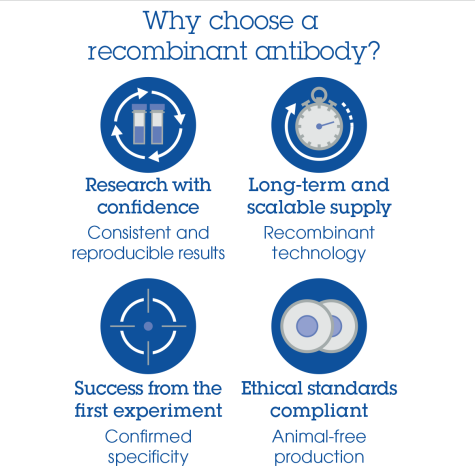


Immunocytochemistry/ Immunofluorescence - Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

This image is courtesy of an Abreview submitted by Francesco Elia Marino

This ICC/IF data was generated using the same anti-Smad3 antibody clone, EP568Y, in a different buffer formulation (cat# **ab40854**).

ab40854 staining Smad3 in human granulosa cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with ethanol and triton and blocked for 1 hour at 26°C. Samples were incubated with primary antibody (1/200) for 16 hours at 4°C. An undiluted IRDye® 800CW-conjugated goat anti-rabbit IgG (H+L) polyclonal was used as the secondary antibody. Left - negative control (4 replicates).



Anti-Smad3 antibody [EP568Y] - BSA and Azide free (ab157372)

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