abcam

ab157371

Anti-Smad2 antibody [EP784Y] - BSA and Azide free

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP784Y
Purification technique	Affinity purification Protein A
Specificity	This antibody is specific for MH 1 domain of Smad2.
Concentration	1.025 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Protein-A purification via MabSelect SuRe

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Human

Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-

Notes	ab199376 - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information SMAD2

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 SMAD2/SMAD4 complex, activates transcription. Promotes TGFB1-mediated transcription of odontoblastic differentiation genes in dental papilla cells (By similarity). Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator. May act as a tumor suppressor in colorectal carcinoma (PubMed:8752209).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab157371 is the carrier-free version of ab40855.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Smad2 also known as Mothers against decapentaplegic homolog 2 (MAD2) or MADR2 is a signaling protein involved in the transforming growth factor-beta (TGF-β) receptor pathway. Smad2 has a molecular mass of approximately 58 kDa and expresses in various tissues including epithelial mesenchymal and endothelial cells. Smad2 undergoes phosphorylation on serine residues in response to TGF-β signaling converting it into phosphorylated forms often referred to as p-Smad2 or phospho-Smad2. These phosphorylated forms are critical for the relay of signals from the cell surface to the nucleus.
Biological function summary	Smad2 acts as an intracellular mediator for TGF-β signaling a pathway important for regulating cell proliferation differentiation and apoptosis. Smad2 typically functions as part of a heteromeric complex with Smad4 another key player in TGF-β signaling. Upon activation phosphorylated Smad2 combines with Smad4 to form a complex that translocates into the nucleus. This complex then binds to specific DNA sequences to regulate the transcription of target genes involved in processes such as cell growth inhibition and extracellular matrix production.
Pathways	Smad2 is integral to the TGF-β and activin receptor signaling pathways. These pathways are essential in controlling cell growth and immune responses. Smad2 interacts with other proteins such as Smad3 in addition to Smad4 to modulate gene expression effectively. The interaction between Smad2 and these proteins ensures precise control of cellular responses to external stimuli emphasizing its pivotal role in maintaining cellular homeostasis.
Associated diseases and disorders	Smad2 correlates with various pathological conditions including fibrosis and cancer. Aberrant Smad2 signaling can contribute to the development of these diseases as excessive TGF-β signaling promotes fibrotic tissue deposition and tumor progression. Smad2 connects with other proteins like Smad3 in these pathological contexts both acting as mediators of abnormal cellular behaviors. Understanding the regulatory mechanisms of Smad2 can help develop therapeutic strategies against disorders linked to dysregulated TGF-β signaling.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

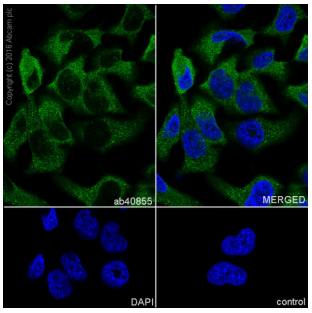
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

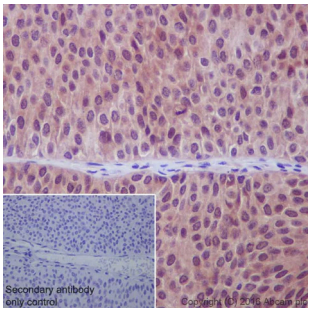
12 product images



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

Smad2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad2 antibody

Immunofluorescence staining of HeLa cells with purified ab40855 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was an Alexa Fluor® 488 conjugated goat anti-rabbit (ab150077), used at a dilution of 1/1000. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative control is shown in bottom right hand panel - for the negative control, PBS was used 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 (ab40855).

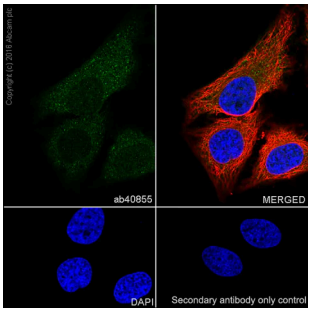


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

Smad2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Smad2 antibody

This IHC data was generated using the same anti-Smad2 antibody clone, EP784Y, in a different buffer formulation (cat# ab40855).
ab40855 stainingSmad2 in human bladder carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/50. A ImmunoHistoProbe one step HRP Polymer was used as a secondary antibody, ready to use.

Negative control 1: PBS in place of primary antibody.

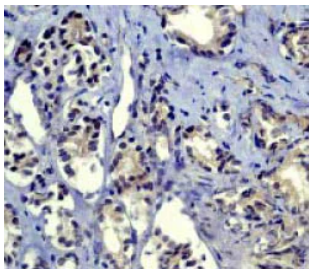


Immunocytochemistry/ Immunofluorescence - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

Smad2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad2 antibody

ab40855 staining Smad2 in HeLa (human cervix adenocarcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary

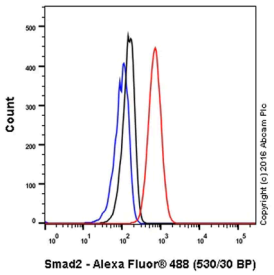
antibody at a dilution of 1/1000. ab195889 was used as a counterstain for primary antibody ab40855 at 1/1000. DAPI was used as a nuclear counterstain and PBS as a negative control. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40855).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

Smad2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Smad2 antibody

ab40855 at a 1:100 dilution staining Smad2 in human prostate carcinoma tissue.This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40855).



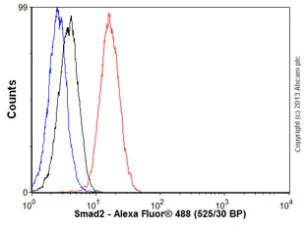
Flow Cytometry (Intracellular) - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

Smad2 Flow Cytometry (Intracellular) staining using rabbit Anti-Smad2 antibody

ab40855 staining Smad2 in the human cell line HeLa (Human epithelial cell line from cervix adenocarcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/20. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.
Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)

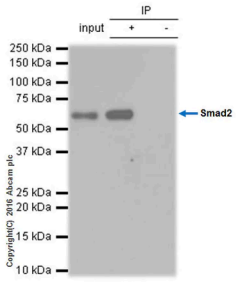
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40855).



Flow Cytometry (Intracellular) - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

Smad2 Flow Cytometry (Intracellular) staining using rabbit Anti-Smad2 antibody

Overlay histogram showing PC3 cells stained with ab40855 (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 (ab40855, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 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. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40855).



Immunoprecipitation - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

ab40855 (purified) at 1/20 immunoprecipitating EGFR in HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate.
Lane 1 (input): HeLa whole cell lysate (10µg)

Lane 2 (+): ab40855 + HeLa whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab40855 in HeLa whole cell lysate.

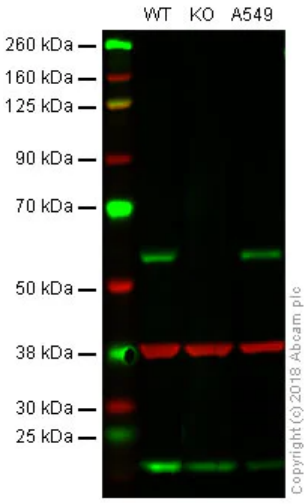
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40855).

All lanes:
Immunoprecipitation - Anti-Smad2 antibody [EP784Y] (ab40855)

Predicted band size: 52 kDa



Western blot - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

Lanes 1 - 3: Merged signal (red and green). Green - ab40855 observed at 58 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab40855 was shown to specifically react with Smad2 in wild-type HeLa cells as signal was lost in Smad2 knockout cells. Wild-type and SMAD2 knockout samples were subjected to SDS-PAGE. ab40855 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/10000 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40855).

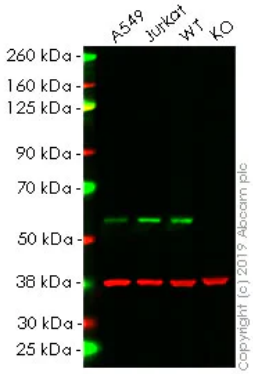
All lanes:
Western blot - Anti-Smad2 antibody [EP784Y] (ab40855) at 1/1000 dilution

Lane 1:
Wild-type HeLa whole cell lysate at 20 µg

Lane 2:
Smad2 knockout HeLa whole cell lysate at 20 µg

Lane 3:
A549 whole cell lysate at 20 µg

Predicted band size: 52 kDa



Western blot - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

Smad2 Western blot staining using rabbit Anti-Smad2 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab40855).
Lanes 1 - 4: Merged signal (red and green). Green - ab40855 observed at 58 kDa. Red - loading control, ab8245 observed at 37 kDa.

ab40855 was shown to react with Smad2 in wild-type HeLa. Loss of signal was observed when knockout cell line ab255430 (knockout cell lysate ab263833) was used. Wild-type and Smad2 knockout samples were subjected to SDS-PAGE. ab40855 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 2000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Smad2 antibody [EP784Y] (ab40855) at 1/2000 dilution

Lane 1:
A549 cell lysate at 20 µg

Lane 2:
Jurkat cell lysate at 20 µg

Lane 2:
Western blot - Human SMAD2 knockout HeLa cell line (ab255430)

Lane 3:
Wild-type HeLa cell lysate at 20 µg

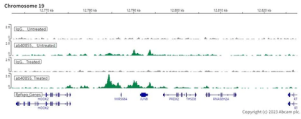
Lane 4:
SMAD2 knockout HeLa cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

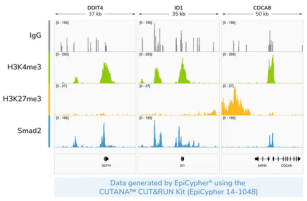
Predicted band size: 52 kDa

Observed band size: 37 kDa, 55 kDa



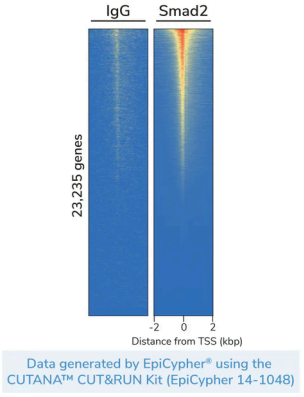
ChIC/CUT&RUN sequencing - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

This data was developed using the same antibody clone in a different buffer formulation (ab40855). ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HaCaT (Human keratinocyte cell line) cells (treated with 7ng/ml TGF-β for 1h) and 5 µg of ab40855 [EP784Y]. 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. Additional screenshots of mapped reads can be found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



ChIC/CUT&RUN sequencing - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

This data was developed using ab40855, the same antibody clone in a different buffer formulation. CUT&RUN profiling with Smad2 antibody reveals the expected genomic enrichment pattern in cells. Representative genome browser tracks show CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with Smad2 antibody (Abcam ab40855, 0.5 µg). 500,000 HeLa cells were used per reaction. IgG, H3K4me3, and H3K27me3 antibodies were included as controls to assess non-specific background, active promoters, and repressed chromatin, respectively. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Images were generated using Integrative Genomics Viewer (IGV, Broad Institute).



ChIC/CUT&RUN sequencing - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

This data was developed using ab40855, the same antibody clone in a different buffer formulation. CUT&RUN profiling with Smad2 antibody demonstrates robust genome-wide enrichment in cells. Heatmaps of genome-wide signal flanking annotated transcription start sites (TSSs, +/- 2 kbp) display CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with Smad2 antibody (Abcam ab40855, 0.5 µg). 500,000 HeLa cells were used per reaction. IgG antibody was included as a negative control to assess non-specific background. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Heatmaps were generated using deepTools (Ramírez et al., Nucleic Acids Res. 2014; PMID: 24799436). Row-linked data are ranked by intensity relative to Smad2, with red indicating high localized enrichment and blue denoting background.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.