abcam

ab33875

Anti-Smad2 antibody [EP567Y]

Recombinant | RabMAb | Advanced Validation | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP567Y
Purification technique	Affinity purification Protein A
Specificity	This antibody detects a region about 40AA before the MH2 region (not the MH2 region itself).
Concentration	0.582 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-

Notes	-

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

IP

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000 - 1/2000
Notes	**For unpurified, use 1/1000.**

Species	Human
Dilution info	1/1000 - 1/2000
Notes	**For unpurified, use 1/1000.**

Predicted

Species	Rat

Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/300
Notes	-

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/110

Notes	For unpurified, use 1/70. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

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

Predicted

Species	Rat
Dilution info	-
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	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C

Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

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

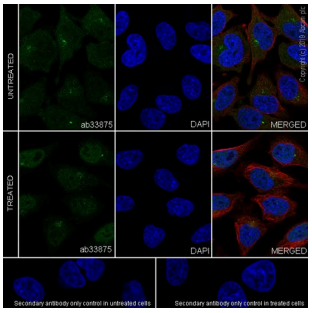
We do not recommend this combination. It is not covered by our product promise.

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.

10 product images

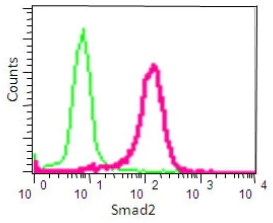


Immunocytochemistry/ Immunofluorescence - Anti-Smad2 antibody [EP567Y] (ab33875)

Smad2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad2 antibody

Immunocytochemistry/Immunofluorescent analysis of HeLa (human cervix adenocarcinoma epithelial) cells labeling Smad2 with ab33875 at a dilution of 1/500. ab150077, an Alexa Fluor® 488 goat anti-rabbit was used at 1/1000 was used as the secondary antibody. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% tritonX-100. Counterstain antibody: ab195889, anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200. Secondary antibody only negative control is shown in the bottom panels.

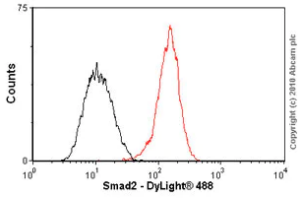
Confocal image showing mainly nuclear staining on HeLa cells after the treatment with TGF-b (10ng/mL) for 1 hour.



Flow Cytometry (Intracellular) - Anti-Smad2 antibody [EP567Y] (ab33875)

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

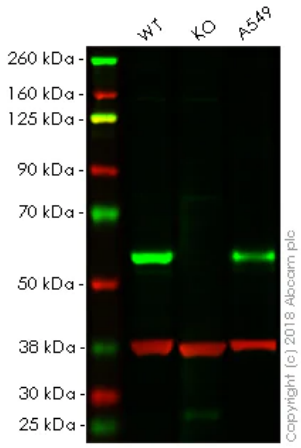
Overlay histogram showingJurkat cells stained with purified ab33875 (pink line) at a dilution of 1/110. The cells were fixed with 2% PFA.FITC goat anti-rabbit was used at a dilution of 1/150 and rabbit monoclonal IgG was used as theisotype control (green line).



Flow Cytometry (Intracellular) - Anti-Smad2 antibody [EP567Y] (ab33875)

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

Overlay histogram showing PC3 cells stained with unpurified ab33875 (red line). The cells were fixed with 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 (ab33875, 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 monoclonal IgG (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a decreased signal in PC3 cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



Western blot - Anti-Smad2 antibody [EP567Y] (ab33875)

Smad2 Western blot staining using rabbit Anti-Smad2 antibody

Lanes 1 - 3: Merged signal (red and green). Green - ab33875 observed at 52 kDa. Red - loading control, ab9484, observed at 37 kDa.
ab33875 was shown to specifically react with Smad2 in wild-type WT HeLa cells as signal was lost in SMAD2 knockout cells. Wild-type and SMAD2 knockout samples were subjected to SDS-PAGE. ab33875 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 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.

All lanes:
Western blot - Anti-Smad2 antibody [EP567Y] (ab33875) at 1/1000 dilution

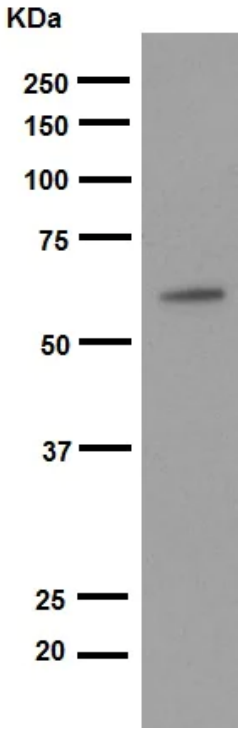
Lane 1:
Wild-type HeLa whole cell lysate at 20 µg

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

Observed band size: 52 kDa



Western blot - Anti-Smad2 antibody [EP567Y] (ab33875)

Smad2 Western blot staining of Jurkat cell lysate using rabbit Anti-Smad2 antibody

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST

All lanes:
Western blot - Anti-Smad2 antibody [EP567Y] (ab33875) at 1/2000 dilution

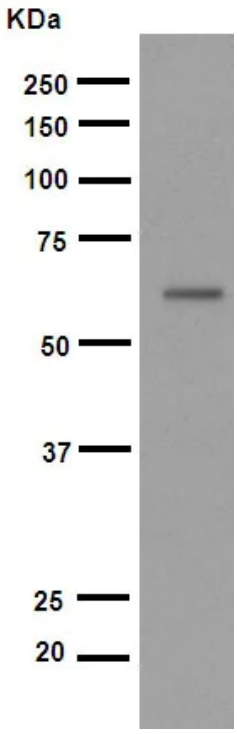
All lanes:
Jurkat cell lysate at 10 µg

Secondary

All lanes:
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 52 kDa

Observed band size: 58 kDa



Western blot - Anti-Smad2 antibody [EP567Y] (ab33875)

Smad2 Western blot staining of Jurkat cell lysate using rabbit Anti-Smad2 antibody

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST

All lanes:
Western blot - Anti-Smad2 antibody [EP567Y] (ab33875) at 1/1000 dilution

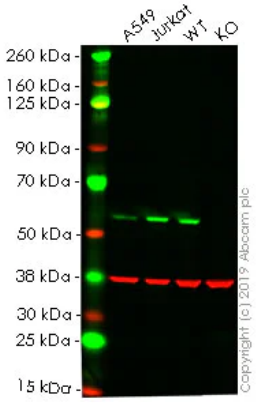
All lanes:
Jurkat cell lysate at 10 µg

Secondary

All lanes:
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 52 kDa

Observed band size: 58 kDa



Western blot - Anti-Smad2 antibody [EP567Y] (ab33875)

Smad2 Western blot staining using rabbit Anti-Smad2 antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab33875 observed at 58 kDa. Red - loading control, ab8245 observed at 37 kDa.
ab33875 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. ab33875 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 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 [EP567Y] (ab33875) at 1/1000 dilution

Lane 1:
Wild-type 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

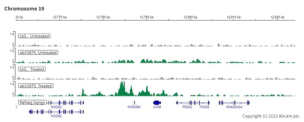
Secondary

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

Performed under reducing conditions.

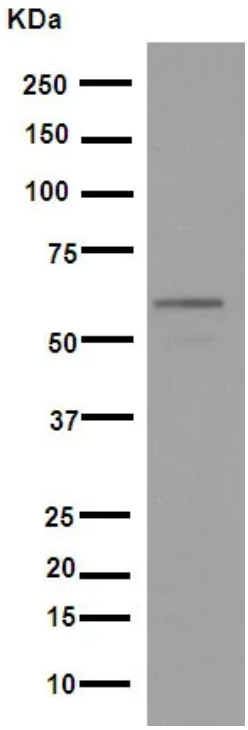
Predicted band size: 52 kDa

Observed band size: 37 kDa, 58 kDa



ChIC/CUT&RUN sequencing - Anti-Smad2 antibody [EP567Y] (ab33875)

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 ab33875 [EP567Y]. 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.



Western blot - Anti-Smad2 antibody [EP567Y] (ab33875)

Smad2 Western blot staining of RAW264.7 using rabbit Anti-Smad2 antibody

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST

All lanes:
Western blot - Anti-Smad2 antibody [EP567Y] (ab33875) at 1/1000 dilution

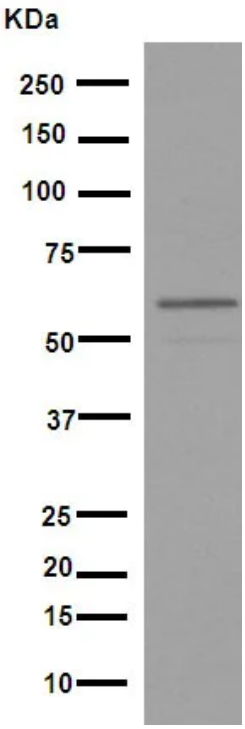
All lanes:
RAW264.7 at 10 µg

Secondary

All lanes:
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 52 kDa

Observed band size: 58 kDa



Western blot - Anti-Smad2 antibody [EP567Y] (ab33875)

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST

All lanes:
Western blot - Anti-Smad2 antibody [EP567Y] (ab33875) at 1/500 dilution

All lanes:
RAW264.7 cell lysate at 10 µg

Secondary

All lanes:
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 52 kDa

Observed band size: 58 kDa

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