abcam

Product datasheet

Anti-Smad2 antibody [EP567Y] ab33875

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Smad2 antibody [EP567Y]
Description	Rabbit monoclonal [EP567Y] to Smad2
Host species	Rabbit
Specificity	This antibody detects a region about 40AA before the MH2 region (not the MH2 region itself).
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIC/CUT&RUN-seq, WB, ICC/IF **Unsuitable for:** IHC-P or IP
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide within Human Smad2 aa 200-300. The exact sequence is proprietary.
Positive control	WB: HeLa, A549, RAW264.7, and Jurkat cell lysate ICC/IF: HeLa cells Flow Cyt (intra): PC3 and Jurkat cells ChIC/CUT&RUN seq: HaCaT cell.
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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP567Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab33875 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
Flow Cyt (Intra)		1/110. For unpurified, use 1/70. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
WB	★★★★☆ **(2)**	1/1000 - 1/2000. Detects a band of approximately 58 kDa (predicted molecular weight: 58 kDa). **For unpurified, use 1/1000.**
ICC/IF		1/300.

Application notes Is unsuitable for IHC-P 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 SMAD2/SMAD4 complex, activates transcription. May act as a tumor suppressor in colorectal carcinoma.

Tissue specificity Expressed at high levels in skeletal muscle, heart and placenta.

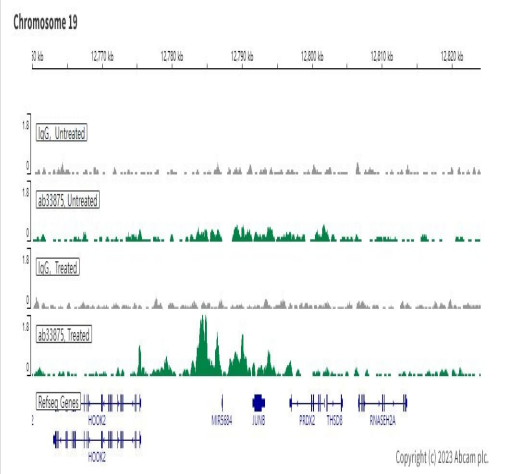
Sequence similarities Belongs to the dwarfin/SMAD family.
Contains 1 MH1 (MAD homology 1) domain.
Contains 1 MH2 (MAD homology 2) domain.

Post-translational modifications Phosphorylated on one or several of Thr-220, Ser-245, Ser-250, and Ser-255. In response to TGF-beta, phosphorylated on Ser-465/467 by TGF-beta and activin type 1 receptor kinases. Able to interact with SMURF2 when phosphorylated on Ser-465/467, recruiting other proteins, such as SNON, for degradation. In response to decorin, the naturally occurring inhibitor of TGF-beta signaling, phosphorylated on Ser-240 by CaMK2. Phosphorylated by MAPK3 upon EGF stimulation; which increases transcriptional activity and stability, and is blocked by calmodulin. In response to TGF-beta, ubiquitinated by NEDD4L; which promotes its degradation. Acetylated on Lys-19 by coactivators in response to TGF-beta signaling, which increases transcriptional activity. Isoform short: Acetylation increases DNA binding activity in vitro and enhances its association with target promoters in vivo. Acetylation in the nucleus by EP300 is enhanced by TGF-beta.

Cellular localization Cytoplasm. Nucleus. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta

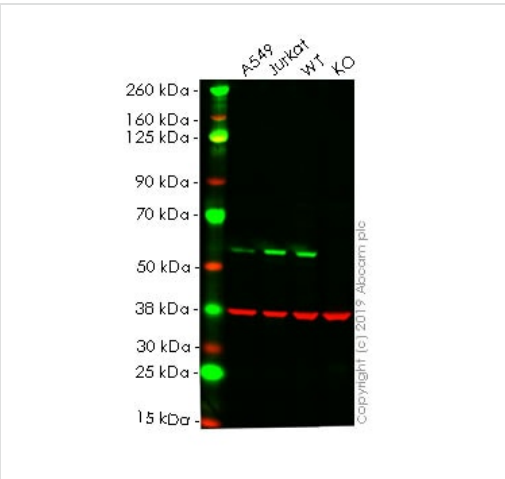
stimulation, migrates to the nucleus when complexed with SMAD4. On dephosphorylation by phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1.

Images



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/µL, 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 downloaded **here**.
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



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

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

Lane 1 : Wild-type A549 cell lysate
Lane 2 : Jurkat cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : SMAD2 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

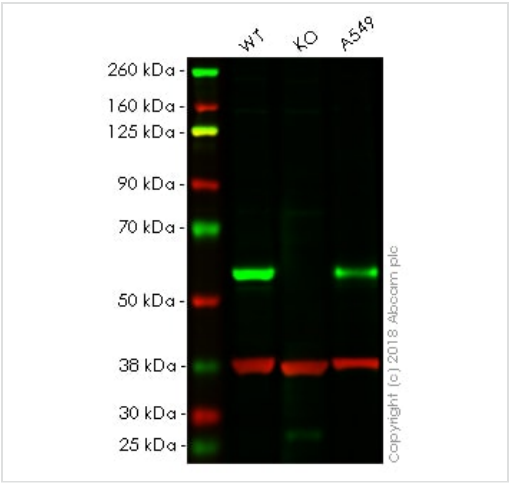
Performed under reducing conditions.

Predicted band size: 58 kDa

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.



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

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

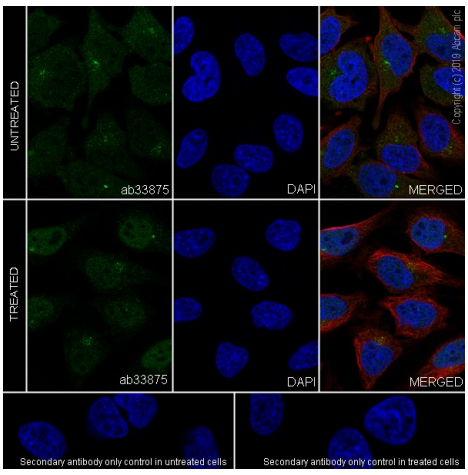
Lane 1 : Wild-type HeLa whole cell lysate
Lane 2 : SMAD2 knockout HeLa whole cell lysate
Lane 3 : A549 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 58 kDa
Observed band size: 52 kDa

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.

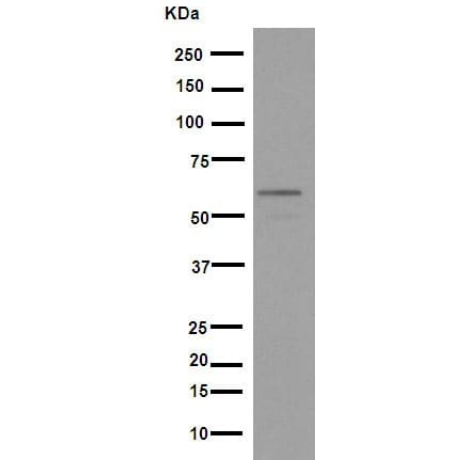


Immunocytochemistry/ Immunofluorescence - Anti-Smad2 antibody [EP567Y] (ab33875)

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.



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

Anti-Smad2 antibody [EP567Y] (ab33875) at 1/1000 dilution (Purified) + RAW264.7 at 10 µg

Secondary

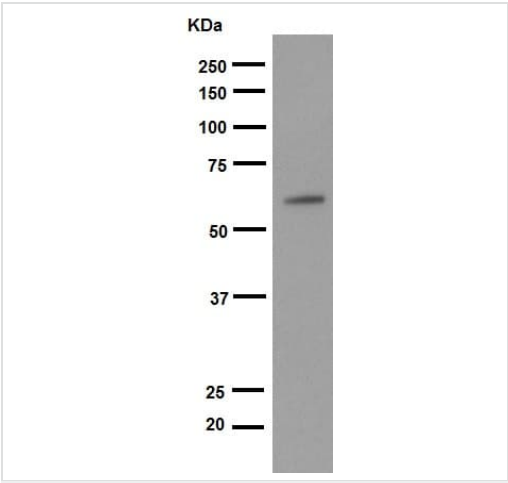
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 58 kDa

Observed band size: 58 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



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

Anti-Smad2 antibody [EP567Y] (ab33875) at 1/2000 dilution (Purified) + Jurkat cell lysate at 10 µg

Secondary

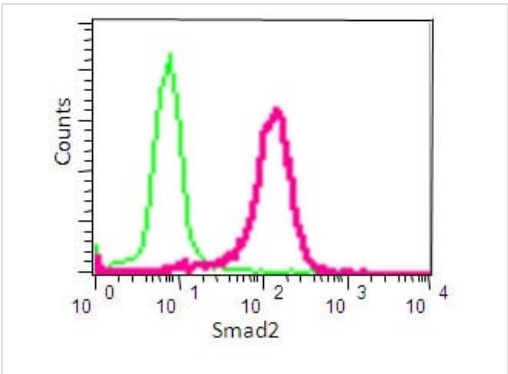
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 58 kDa

Observed band size: 58 kDa

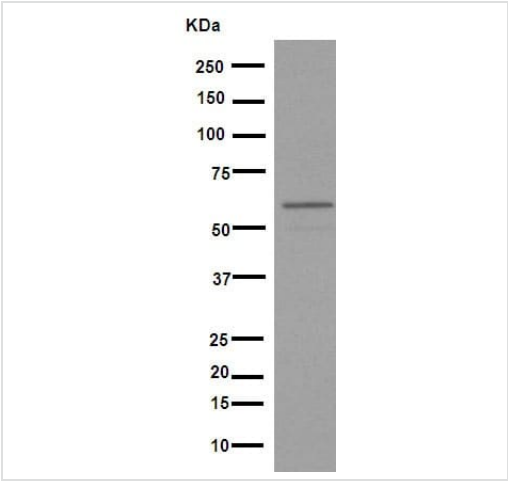
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



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

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).



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

Anti-Smad2 antibody [EP567Y] (ab33875) at 1/500 dilution + RAW264.7 cell lysate at 10 µg

Secondary

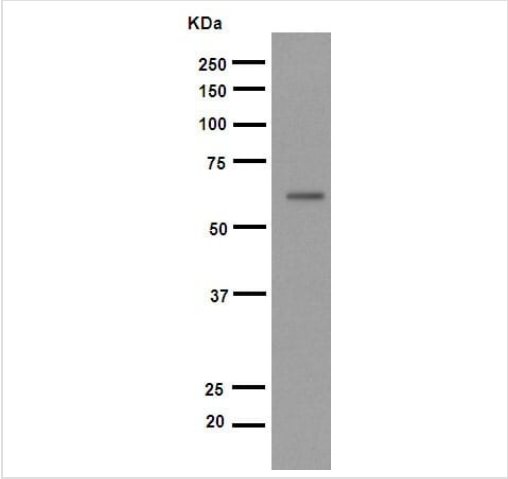
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 58 kDa

Additional bands at: 58 kDa. We are unsure as to the identity of these extra bands.

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



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

Anti-Smad2 antibody [EP567Y] (ab33875) at 1/1000 dilution (Unpurified) + Jurkat cell lysate at 10 µg

Secondary

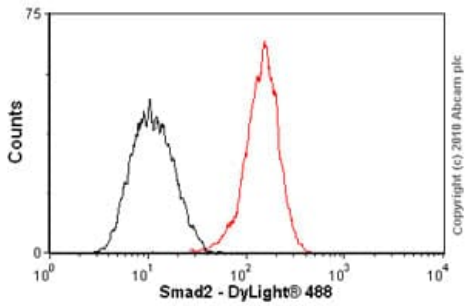
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 58 kDa

Additional bands at: 58 kDa. We are unsure as to the identity of these extra bands.

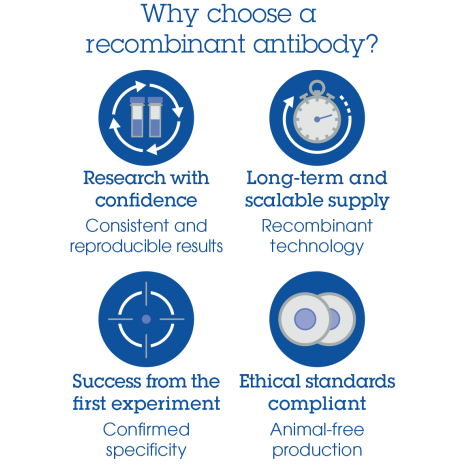
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



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

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.



Anti-Smad2 antibody [EP567Y] (ab33875)

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