abcam

Product datasheet

Anti-Smad2 antibody [EP784Y] ab40855

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Smad2 antibody [EP784Y]
Description	Rabbit monoclonal [EP784Y] to Smad2
Host species	Rabbit
Specificity	This antibody is specific for MH 1 domain of Smad2.
Tested applications	**Suitable for:** IHC-P, IP, ICC/IF, Flow Cyt (Intra), ChIC/CUT&RUN-seq, WB
Species reactivity	**Reacts with:** Rat, Human
Immunogen	Synthetic peptide within Human Smad2 aa 50-150. The exact sequence is proprietary.
Positive control	WB: A549, Jurkat, HeLa, A-673, HUVEC and C6 cell lysates. IP: HeLa IHC-P: Human bladder and prostate carcinoma tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa and PC3 cells. ChIC/CUT&RUN seq: HaCaT cell
General notes	**The rat recommendation is based on the WB results. This antibody may not be suitable for IHC with rat samples.** 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**. Mouse: 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: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP784Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40855 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
IHC-P		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/20 - 1/50.
ICC/IF		1/100 - 1/250.
Flow Cyt (Intra)		1/20 - 1/100. **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	★★★★★ (4)	1/2000 - 1/10000. Detects a band of approximately 55 kDa (predicted molecular weight: 58 kDa).

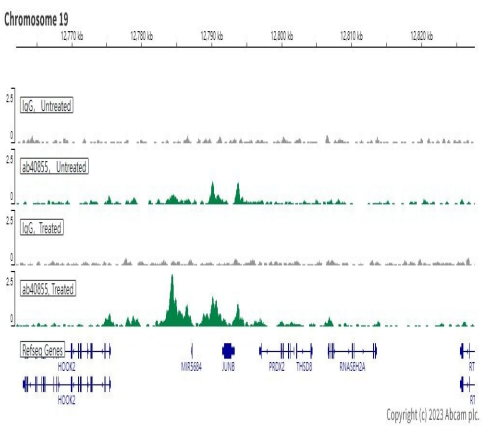
Target

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.

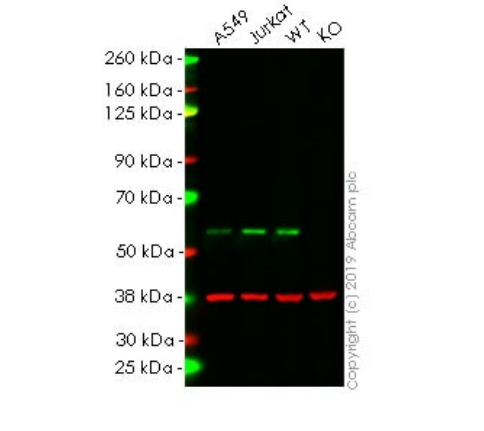
Cellular localization Cytoplasm. Nucleus. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4. On dephosphorylation by phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1.

Images



ChIC/CUT&RUN sequencing - Anti-Smad2 antibody [EP784Y] (ab40855)

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



Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

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

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

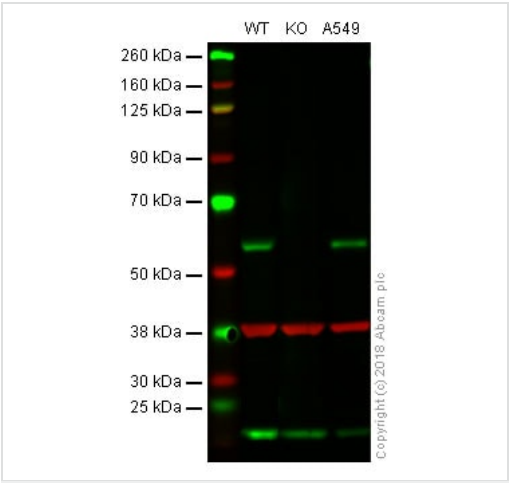
Lysates/proteins at 20 µg per lane.

Predicted band size: 58 kDa

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.



Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

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

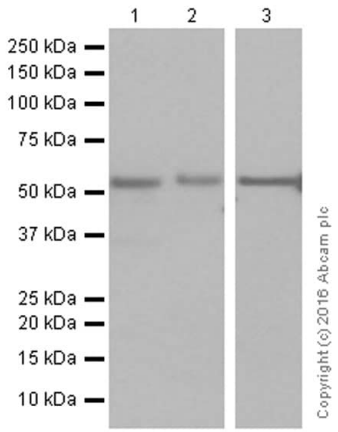
Lane 1 : Wild-type HeLa whole cell lysate
Lane 2 : Smad2 knockout HeLa whole cell lysate
Lane 3 : A549 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 58 kDa

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.



Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

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

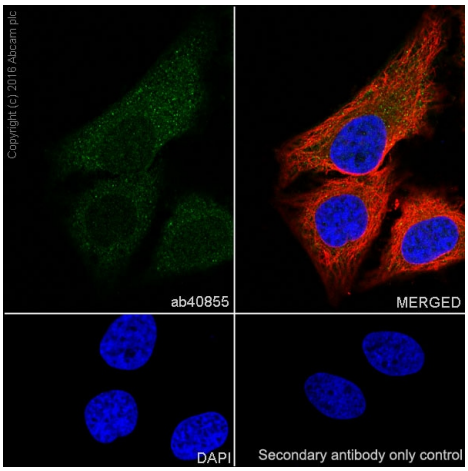
Lane 1 : A-673 (Human muscle Ewing's Sarcoma cell line) whole cell lysate
Lane 2 : HUVEC (Human umbilical vein endothelial cell line) whole cell lysate
Lane 3 : C6 (Rat glial tumor cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

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

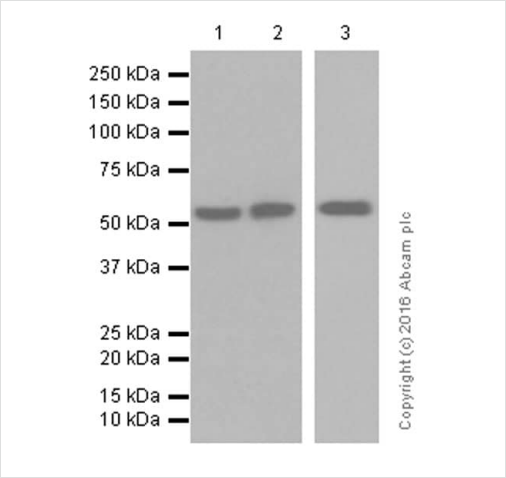
Predicted band size: 58 kDa

Diluting and blocking buffer: 5% NFDM /TBST



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 antibody [EP784Y] (ab40855)

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.



Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

All lanes : Anti-Smad2 antibody [EP784Y] (ab40855) at 1/10000 dilution

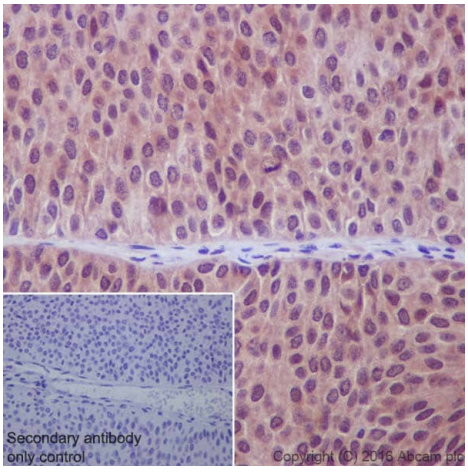
Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lanes 2-3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 20000 µg (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 58 kDa

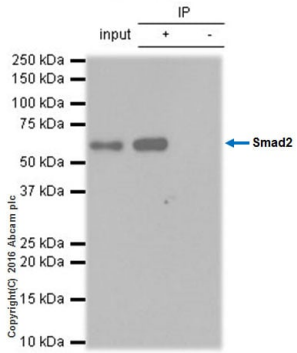
Blocking and diluting buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 antibody [EP784Y] (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.



Immunoprecipitation - Anti-Smad2 antibody [EP784Y] (ab40855)

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

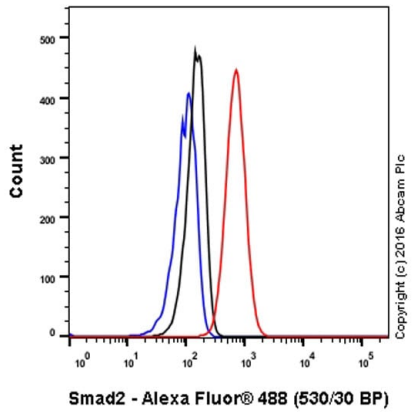
Lane 1 (input): HeLa whole cell lysate (10µg)

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

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

For western blotting, **ab131366** VeriBlot for IP (HRP) was used for detection (1/1000).

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

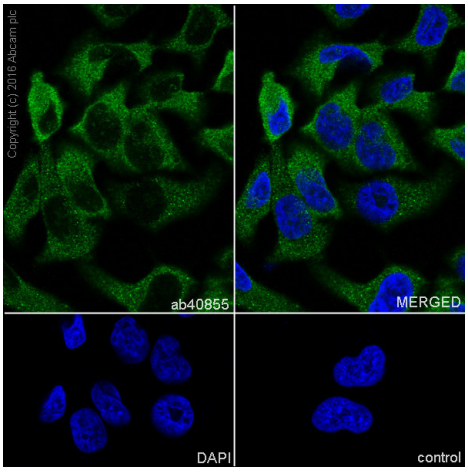


Flow Cytometry (Intracellular) - Anti-Smad2 antibody [EP784Y] (ab40855)

ab40855 staining Smad2in 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.

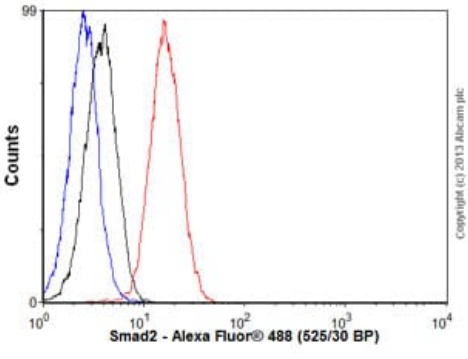
Isoytype control: Rabbit monoclonal IgG (Black)

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



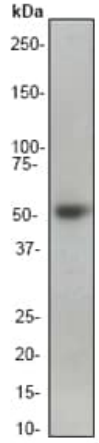
Immunocytochemistry/ Immunofluorescence - Anti-Smad2 antibody [EP784Y] (ab40855)

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.



Flow Cytometry (Intracellular) - Anti-Smad2 antibody [EP784Y] (ab40855)

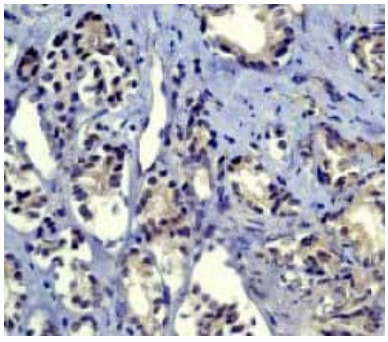
Overlay histogram showing PC3 cells stained with ab40855 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions 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.



Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

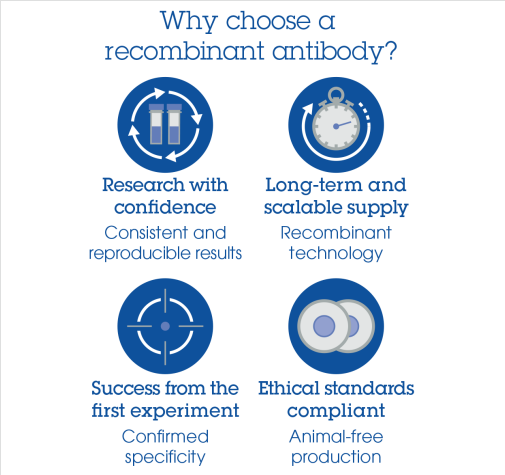
Anti-Smad2 antibody [EP784Y] (ab40855) at 1/500000 dilution + Jurkat cell lysate

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 antibody [EP784Y] (ab40855)

ab40855 at a 1:100 dilution staining Smad2 in human prostate carcinoma tissue.



Anti-Smad2 antibody [EP784Y] (ab40855)

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