abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

11 Images

Overview

Product name	Anti-Smad2 antibody [EP784Y] - BSA and Azide free
Description	Rabbit monoclonal [EP784Y] to Smad2 - BSA and Azide free
Host species	Rabbit
Specificity	This antibody is specific for MH 1 domain of Smad2.
Tested applications	**Suitable for:** ICC/IF, IHC-P, IP, WB, ChIC/CUT&RUN-seq, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Jurkat, A549 and HeLa cell lysates. ICC/IF: HeLa cells. IHC-P: Human prostate carcinoma and human bladder carcinoma tissue. IP: HeLa cell lysate. Flow: HeLa and PC3 cells. ChIC/CUT&RUN seq: HaCaT cell
General notes	ab157371 is the carrier-free version of **ab40855**. 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. 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	EP784Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab157371 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

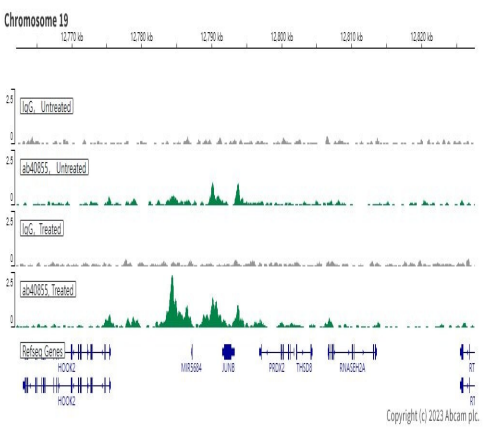
Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 55 kDa (predicted molecular weight: 58 kDa).
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

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	Phosphorylated on one or several of Thr-220, Ser-245, Ser-250, and Ser-255. In response to

modifications	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.

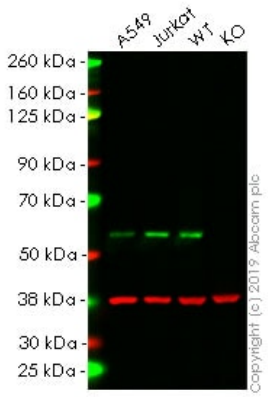
Images



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

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

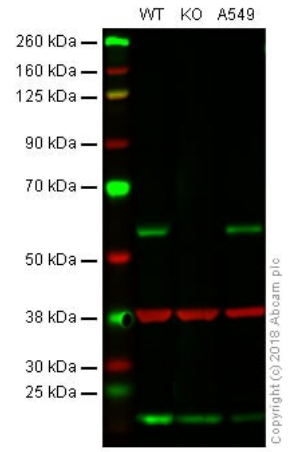
Lysates/proteins at 20 µg per lane.

Predicted band size: 58 kDa
Observed band size: 55 kDa

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.



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

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

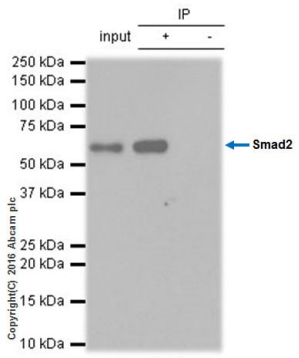
Lysates/proteins at 20 µg per lane.

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.

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)

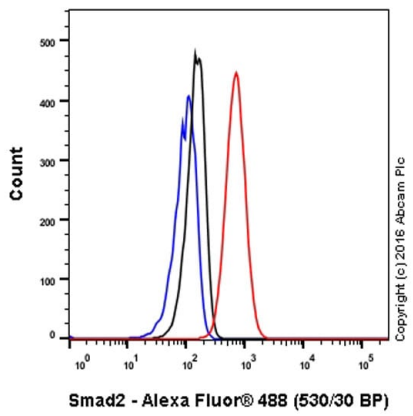
Lane 2 (+): **ab40855** + HeLa whole cell lysate.

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**).



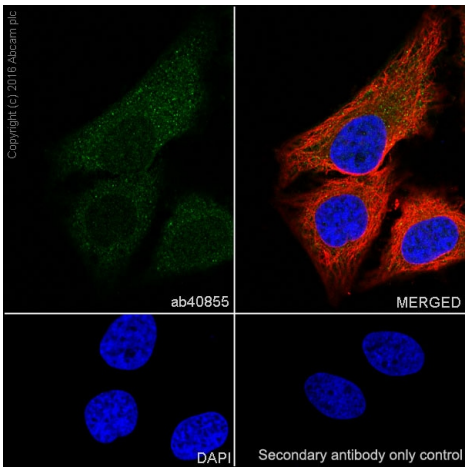
Flow Cytometry (Intracellular) - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

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)

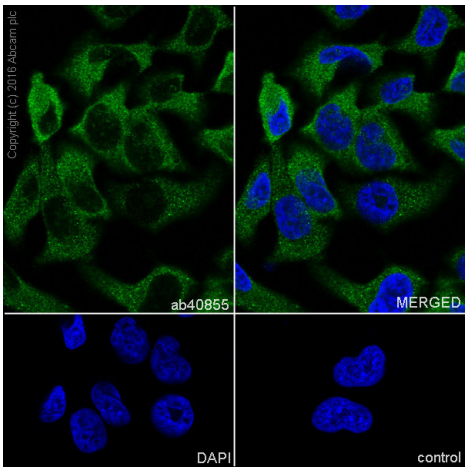
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40855**).



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

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.

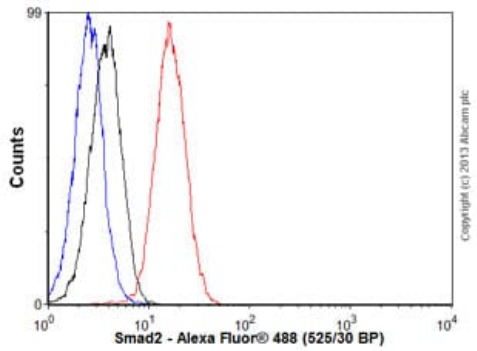
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40855**).



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

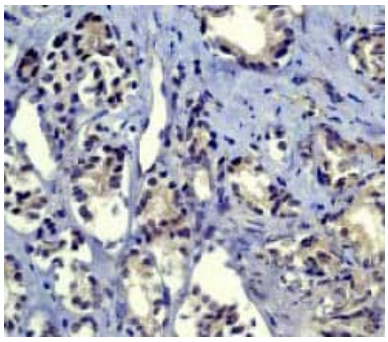
Immunofluorescence staining of HeLa cells with purified **ab40855** at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was an Alexa Fluor® 488 conjugated goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. The cells were fixed in 4% PFA and permeabilized using 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**).



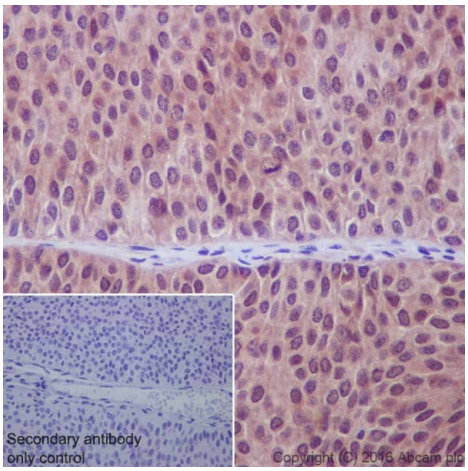
Flow Cytometry (Intracellular) - Anti-Smad2 antibody [EP784Y] - BSA and Azide free (ab157371)

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**).



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

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**).



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

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.

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

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