abcam

Product datasheet

Anti-Smad2 antibody [EP784Y] ab40855

敲除验证 重组 RabMAb

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概述

产品名称	Anti-Smad2抗体[EP784Y]
描述	兔单克隆抗体[EP784Y] to Smad2
宿主	Rabbit
特异性	This antibody is specific for MH 1 domain of Smad2.
经测试应用	**适用于:** IHC-P, IP, ICC/IF, WB, Flow Cyt
种属反应性	**与反应:** Rat, Human
免疫原	Synthetic peptide within Human Smad2 aa 50-150. The exact sequence is proprietary.
阳性对照	Jurkat cell lysate and human prostate carcinoma tissue.
常规说明	**The rat recommendation is based on the WB results. This antibody may not be suitable for IHC with rat samples.** Mouse: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.** This product is a recombinant rabbit monoclonal antibody.

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
纯度	Protein A purified

克隆	单克隆
克隆编号	EP784Y
同种型	IgG

应用

Our Abpromise guarantee covers the use of **ab40855** in the following tested applications.

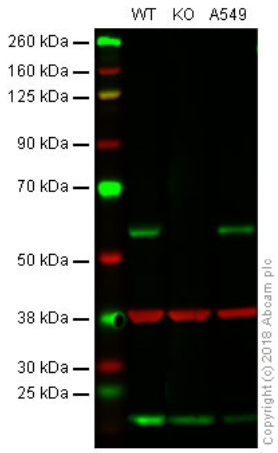
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

应用	Ab评论	说明
IHC-P		1/50.
IP		1/20 - 1/50.
ICC/IF		1/100 - 1/250.
WB	★★★★★	1/2000 - 1/10000. Detects a band of approximately 55 kDa (predicted molecular weight: 58 kDa).
Flow Cyt		1/20 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

靶标

功能	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.
组织特异性	Expressed at high levels in skeletal muscle, heart and placenta.
序列相似性	Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.
翻译后修饰	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.
细胞定位	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.

图片



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

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

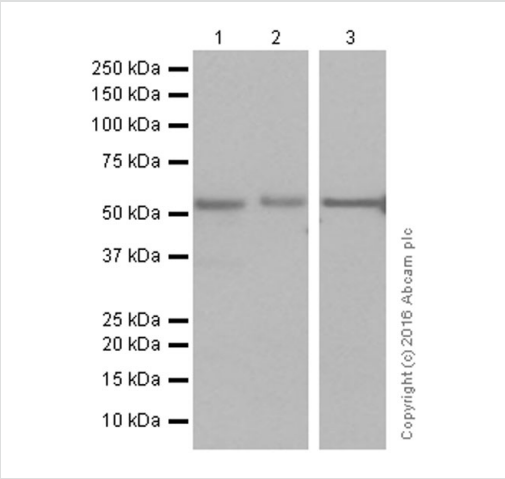
Lane 1 : Wild-type HeLa whole cell lysate
Lane 2 : Smad2 knockout HeLa whole cell lysate
Lane 3 : A549 whole cell lysate

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

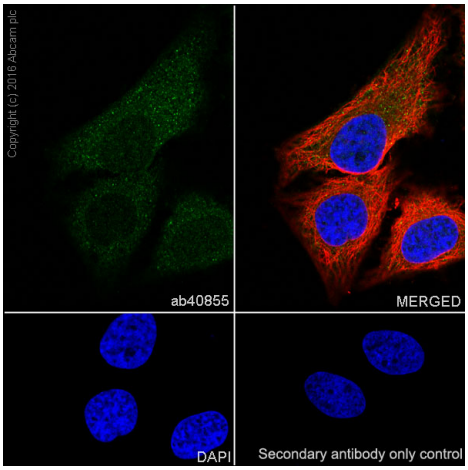
Lane 1 : A-673 (Human muscle Ewing's Sarcoma cell line) whole cell lysate
Lane 2 : HUVEC (Human umbilical vein endothelial cell line) whole cell lysate
Lane 3 : C6 (Rat glial tumor cell line) whole cell lysate

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)

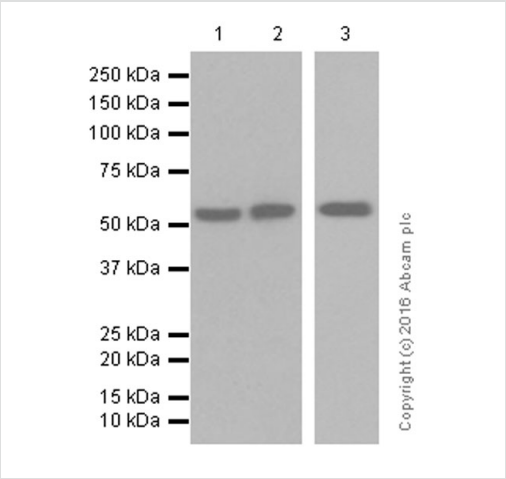
Predicted band size: 58 kDa

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

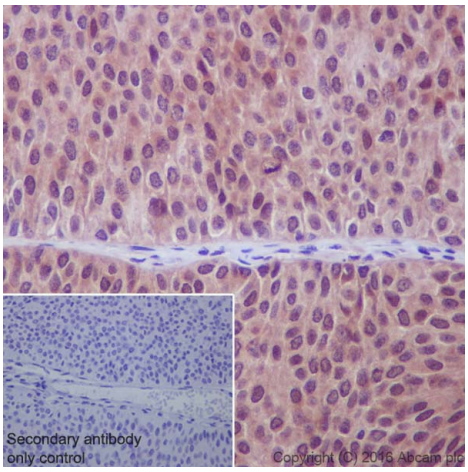
Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lanes 2-3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

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

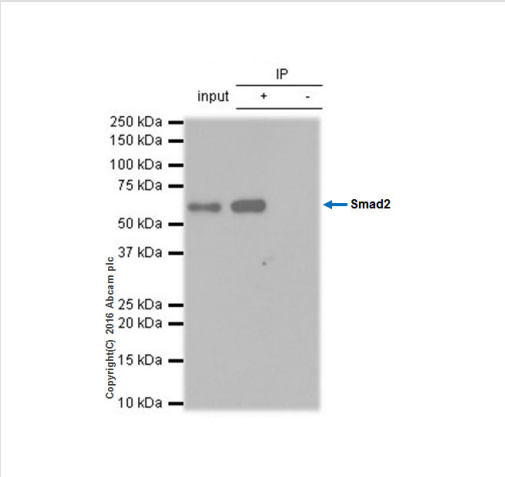
Blocking and diluting buffer: 5% NFDM/TBST



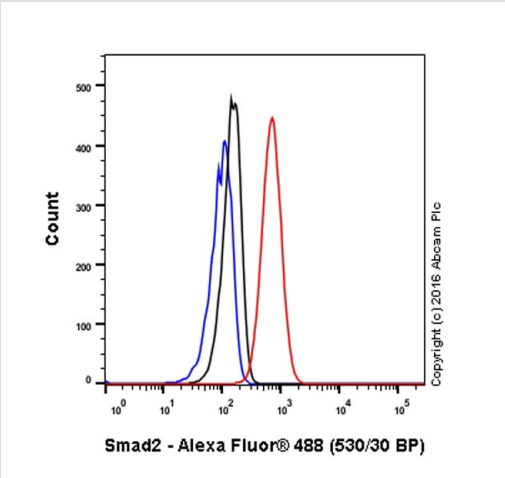
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 antibody [EP784Y] (ab40855)

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)

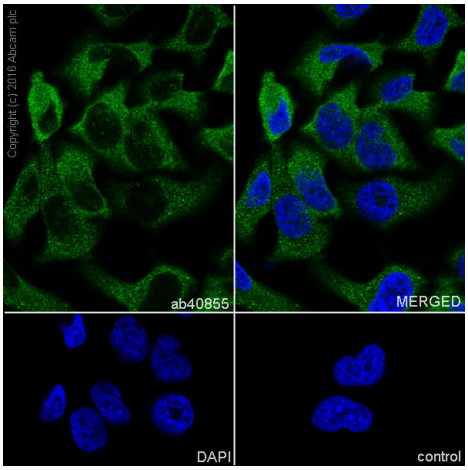


Flow Cytometry - Anti-Smad2 antibody [EP784Y] (ab40855)

ab40855 staining Smad2 in the human cell line HeLa (Human epithelial cell line from cervix adenocarcinoma) by 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.

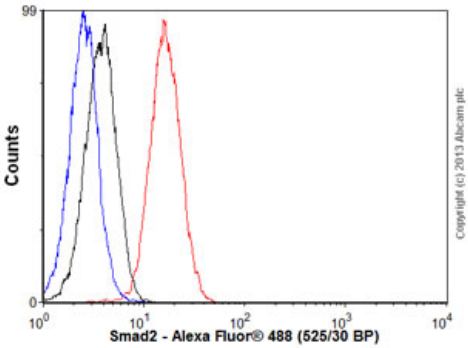
Isoytype control: Rabbit monoclonal IgG (Black)

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



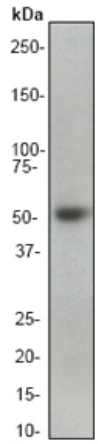
Immunocytochemistry/ Immunofluorescence - Anti-Smad2 antibody [EP784Y] (ab40855)

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 - Anti-Smad2 antibody [EP784Y] (ab40855)

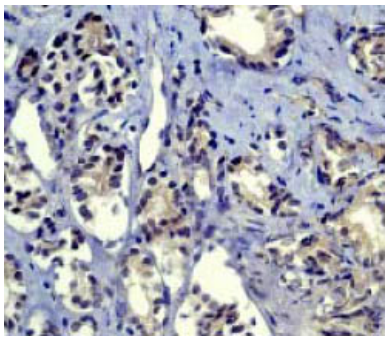
Overlay histogram showing PC3 cells stained with ab40855 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions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

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