abcam

Product datasheet

Anti-Smad2 (phospho S255) antibody [EPR2856(N)] ab188334

Recombinant RabMAb

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Overview

Product name	Anti-Smad2 (phospho S255) antibody [EPR2856(N)]
Description	Rabbit monoclonal [EPR2856(N)] to Smad2 (phospho S255)
Host species	Rabbit
Tested applications	**Suitable for:** Dot blot, IHC-P, IP, WB, ChIC/CUT&RUN-seq
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	Hela treated with Okadaic acid and Calyculin A, Hela treated with Okadaic acid and Calyculin A, Human endometrium, Human transitional cell carcinoma of bladder. RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) and PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates. ChIC/CUT&RUN-Seq: HaCaT cells.
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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR2856(N)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab188334 in the following tested applications.

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

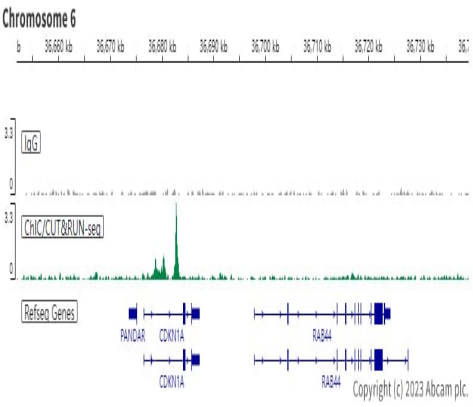
Application	Abreviews	Notes
Dot blot		Use at an assay dependent concentration.
IHC-P	★★★★★ (1)	1/50 - 1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/50 - 1/70.
WB		1/1000 - 1/10000. Detects a band of approximately 58 kDa (predicted molecular weight: 52 kDa).
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg

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

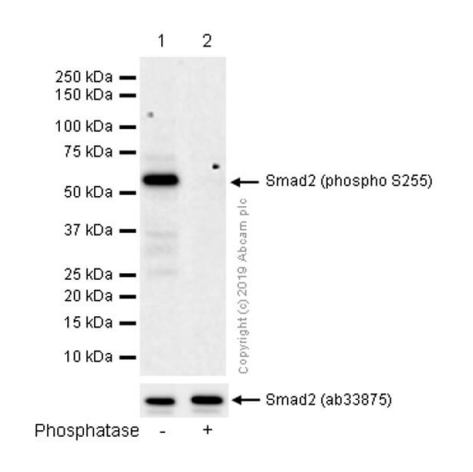


ChIC/CUT&RUN sequencing - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 HaCaT (human skin keratinocyte) cells and 5µg of ab188334 [EPR2856(N)]. 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 (phospho S255) antibody [EPR2856(N)] (ab188334)

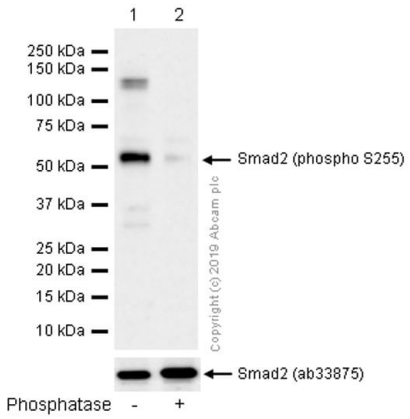
All lanes : Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334) at 1/10000 dilution

Lane 1 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate
Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate, Then the membrane was incubated with phosphatase

Lysates/proteins at 15 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: 52 kDa



Western blot - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

All lanes : Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334) at 1/10000 dilution

Lane 1 : RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 2 : RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate. Then the membrane was incubated with phosphatase.

Lysates/proteins at 15 µg per lane.

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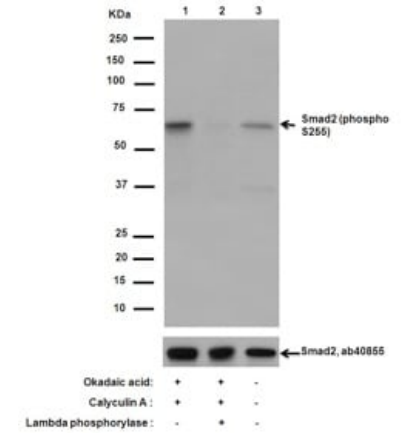


Western blot - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

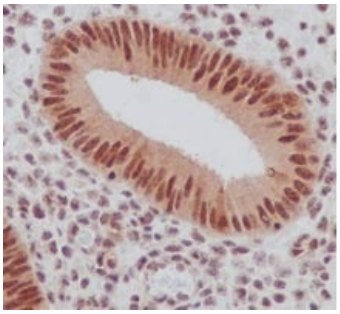
All lanes : Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334) at 1/10000 dilution

Lane 1 : Hela treated with Okadaic acid and Calyculin A
Lane 2 : Hela treated with Okadaic acid and Calyculin A , then treated with Lambda phosphorylase
Lane 3 : Untreated Hela

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

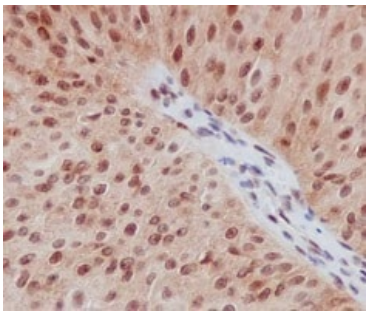
Predicted band size: 52 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

Immunohistochemical analysis of formalin fixed paraffin embedded Human endometrium labeling Smad2 (phospho S255) with ab188334 at 1/100 dilution and HRP Polymer for Rabbit IgG. Counterstained with Hematoxylin.

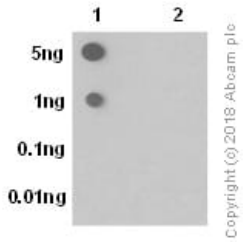
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

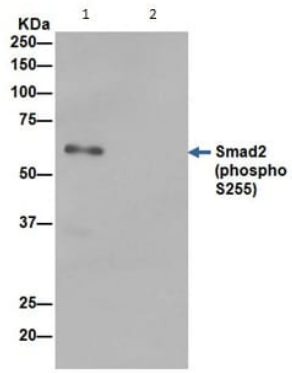
Immunohistochemical analysis of formalin fixed paraffin embedded Human transitional cell carcinoma of bladder labeling Smad2 (phospho S255) with ab188334 at 1/100 dilution and HRP Polymer for Rabbit IgG. Counterstained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Dot Blot - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

Dot blot analysis of Smad2 (S255) phospho peptide (Lane 1), Smad2 non-phospho peptide (Lane 2), labelling Smad2 (S255) phospho peptide with ab188334 at a dilution of 1:1000 dilution (1.365ug/ml). A Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) was used as the secondary antibody at a dilution of 1:20,000 dilution. Blocking buffer: 5% NFDM/TBST. Dilution buffer: 5% NFDM /TBST .



Immunoprecipitation - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

Immunoprecipitation of Hela cells treated with Okadaic acis and Calyculin A (Lane 1) or PBS (Lane 2) labeling Smad2 (phospho S255) with ab188334 at 1/50 dilution and Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1500 dilution

Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

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