abcam

Product datasheet

Anti-Smad2 (phospho S255) antibody [EPR2856(N)] - BSA and Azide free ab219598

Recombinant RabMAb

1 References 6 Images

Overview

Product name Anti-Smad2 (phospho S255) antibody [EPR2856(N)] - BSA and Azide free

Description Rabbit monoclonal [EPR2856(N)] to Smad2 (phospho S255) - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: IP, WB, IHC-P, ChIC/CUT&RUN-seq, Dot blot

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. ChlC/CUT&RUN-Seq: HaCaT cells.

General notes ab219598 is the carrier-free version of <u>ab188334</u>.

Our <u>carrier-free</u> 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 <u>conjugation kits</u> 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.

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

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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 pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR2856(N)

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab219598 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
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 58 kDa (predicted molecular weight: 52 kDa).
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.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
Dot blot		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.

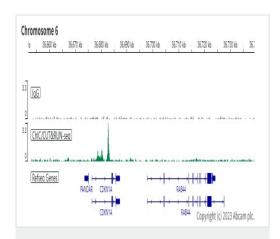
Post-translational modifications

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



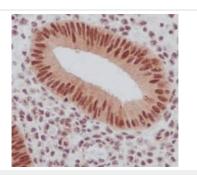
ChIC/CUT&RUN sequencing - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] - BSA and Azide free (ab219598)

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 <u>ab188334</u> [EPR2856(N)]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control <u>ab172730</u> is also shown.

Additional screenshots of mapped reads can be downloaded here.

The University of Geneva owns patents relevant to ChlC (Chromatin Immuno-Cleavage) methods.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab188334**).

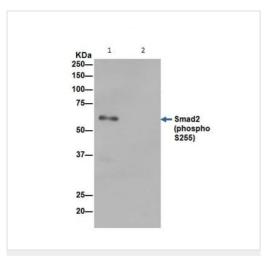


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] - BSA and Azide free (ab219598)

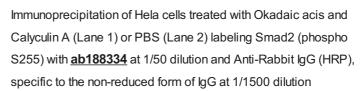
Immunohistochemical analysis of formalin fixed paraffin embedded Human endometrium labeling Smad2 (phospho S255) with ab188334 at 1/100 dilution and HRP Polymer for Rabbit IgG. Counterstained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188334).

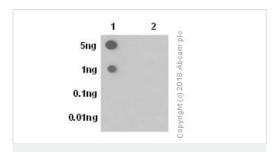
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] - BSA and Azide free (ab219598)



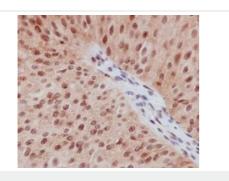
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188334).



Dot Blot - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] - BSA and Azide free (ab219598)

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 lgG, (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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188334).

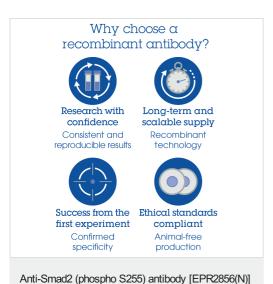


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] - BSA and Azide free (ab219598)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188334).

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



- BSA and Azide free (ab219598)

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