abcam

Product datasheet

Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade ab207447

Recombinant RabMAb

7 References 11 Images

Overview

Product name	Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade
Description	Rabbit monoclonal [EPR19557] to Smad2 + Smad3 - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIC/CUT&RUN-seq, ICC/IF, IP, ChIP, WB
Species reactivity	**Reacts with:** Human, Recombinant fragment
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Recombinant protein fragment human Smad2; recombinant protein fragment human Smad3; HeLa, K562, A549 and Jurkat whole cell lysates; human fetal liver lysate. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. IP: HeLa whole cell lysate. ChIP: Chromatin was prepared from HaCaT cells treated with 7ng/ml TGF-ÃŸ for 1h. ChIC/CUT&RUN seq: HaCaT cell.
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: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR19557
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab207447 in the following tested applications.

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

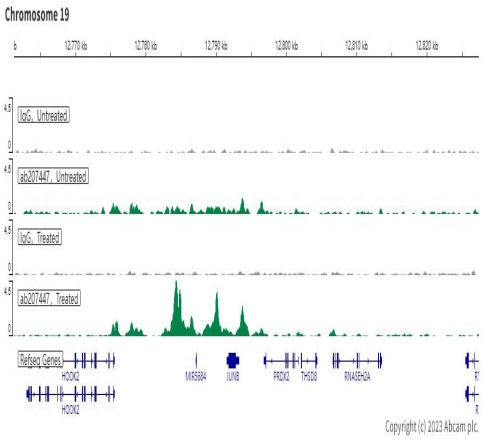
Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
ICC/IF		1/500.
IP		1/40.
ChIP		Use 2 µg for 25 µg of chromatin.
WB		1/2000. Detects a band of approximately 58-62 kDa (predicted molecular weight: 52 kDa).

Target

Relevance SMAD is a family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (Mad) and the *C. elegans* gene Sma. SMAD proteins are signal transducers and transcriptional modulators that mediate multiple signaling pathways. They mediate the signal of the transforming growth factor (TGF)-beta, and thus regulate multiple cellular processes, such as cell proliferation, apoptosis, and differentiation.

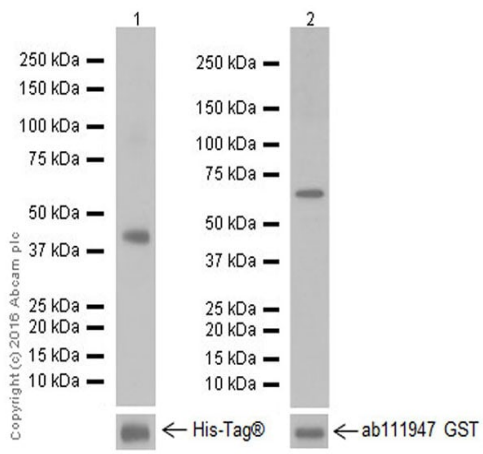
Cellular localization Cytoplasm. Nucleus. Note: Cytoplasmic in the absence of ligand. Migrates to the nucleus when complexed with SMAD4.

Images



ChIC/CUT&RUN sequencing - Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447)

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 ab207447 [EPR19557]. 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 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447)

All lanes : Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447) at 1/50000 dilution

Lane 1 : Recombinant protein fragment human Smad2
Lane 2 : Recombinant protein fragment human Smad3

Lysates/proteins at 0.01 µg per lane.

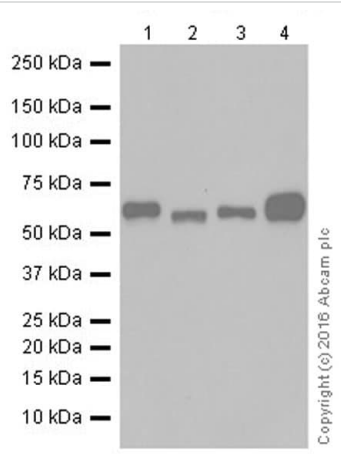
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 52 kDa
Observed band size: 38,60 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 2 seconds; Lane 2: 15 seconds.

Human Smad2 fragment recombinant protein contains aa2-270 with His-Tag®. Human Smad3 fragment recombinant protein contains aa2-227 with His-Tag® and GST-tag.



Western blot - Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447)

All lanes : Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447) at 1/10000 dilution

Lane 1 : HeLa (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lane 2 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate
Lane 3 : A549 (Human lung carcinoma cell line) whole cell lysate
Lane 4 : Human fetal liver lysate

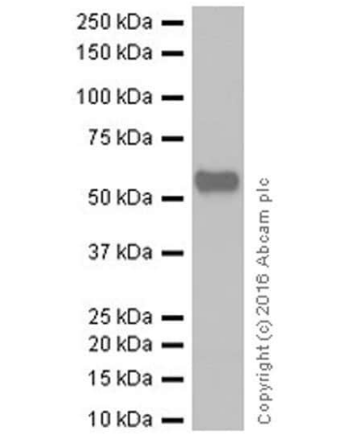
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 52 kDa
Observed band size: 58-62 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447)

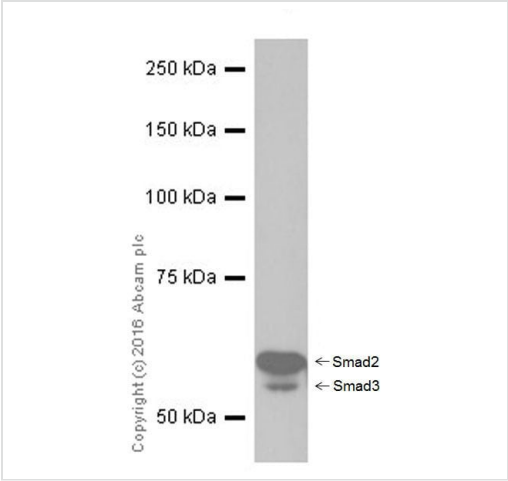
Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447) at 1/2000 dilution + Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate at 10 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 52 kDa
Observed band size: 58-62 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447)

Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447) at 1/10000 dilution + HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 10 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

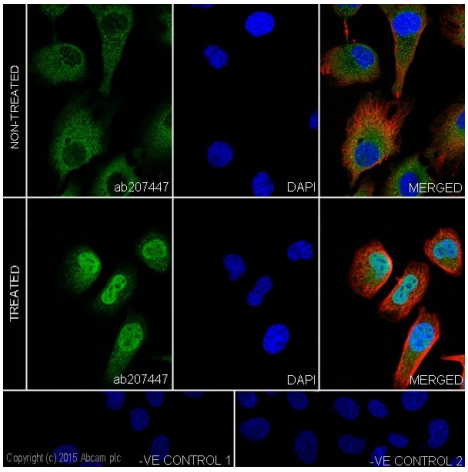
Predicted band size: 52 kDa

Observed band size: 52,58-62 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

Smad2 and Smad3 can be resolved using a lower percentage gel.



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447)

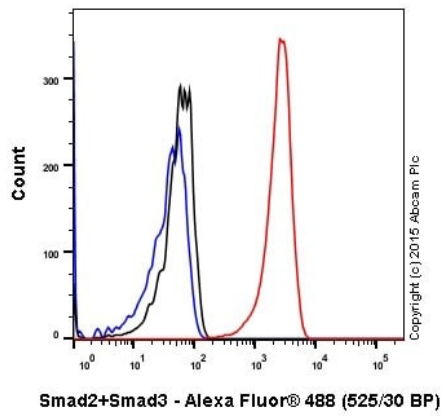
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Smad2 + Smad3 with ab207447 at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing signal translocation from cytoplasm to nucleus after TGF-beta (10ng/ml, 1h) treatment in HeLa cells. PMID: 9006934. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

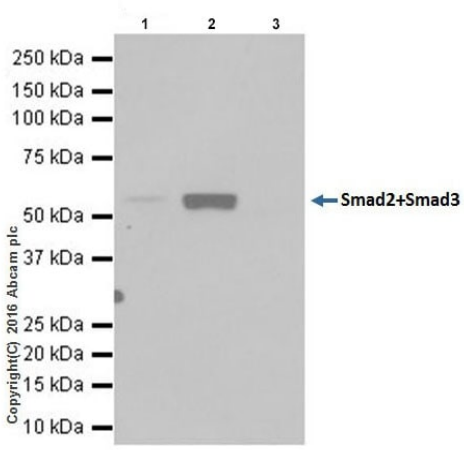
-ve control 1: ab207447 at 1/500 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Smad2 and Smad3with ab207447 at 1/500 dilution (red) compared with a rabbit monoclonal IgG isotype control (**ab172730**; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluorr® 488) at 1/500 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447)

Smad2 + Smad3 were immunoprecipitated from 1mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab207447 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab207447 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

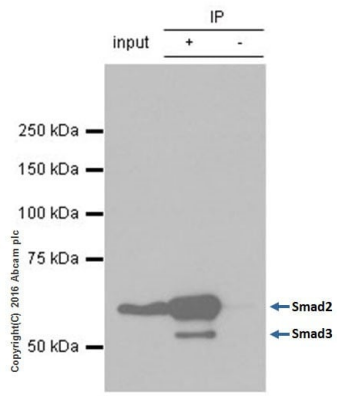
Lane 1: HeLa whole cell lysate 10µg (Input).

Lane 2: ab207447 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab207447 in HeLa whole cell lysate.

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

Exposure time: 5 seconds.



Immunoprecipitation - Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447)

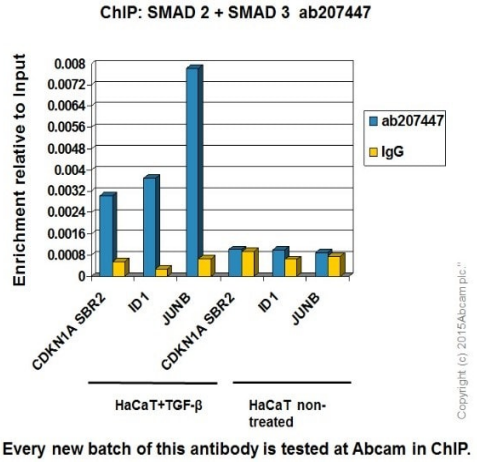
ab207447 Immunoprecipitating Smad2 + Smad3 in human HeLa whole cell lysate . 2µg of capture antibody in 0.35mg lysate was used. 10µg of cell lysate was incubated with primary antibody at a dilution of 1/1000 and VeriBlot for IP Detection Reagent (HRP) **ab131366** was used for detection at a dilution of 1/1000.

Lane 1: HeLa (human cervix adenocarcinoma) whole cell lysate
Lane 2: HeLa (human cervix adenocarcinoma) whole cell lysate
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab207447 in HeLa (human cervix adenocarcinoma) whole cell lysate

Blocking buffer: 5% NFDM/TBST

Diluting buffer: 5% NFDM/TBST

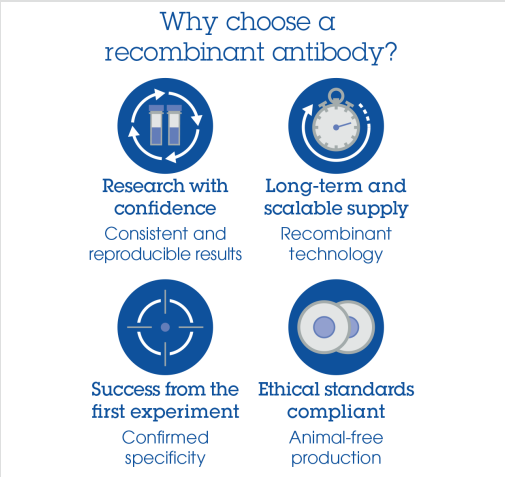
Smad2 and Smad3 can be resolved using a lower percentage gel.



ChIP - Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447)

Chromatin was prepared from HaCaT (Human keratinocyte cell line) cells treated with 7ng/ml TGF-β for 1h and non-treated according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab207447 (blue), and 20µl of Anti rabit IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

The ChIP condition is designed against Smad2 refer to PMID: 18955504.



Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447)

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