abcam

ab207447

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

Recombinant | RabMAb | Advanced Validation | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR19557
Purification technique	Affinity purification Protein A
Concentration	0.53 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/40
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

ChIP

Tested

Species	Human
Dilution info	2 µg for 25 µg chromatin
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

WB

Tested

Species	Recombinant fragment - Human
Dilution info	1/50000
Notes	-

Species	Human
Dilution info	1/2000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

Target data

See full target information SMAD2

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. Promotes TGFB1-mediated transcription of odontoblastic differentiation genes in dental papilla cells (By similarity). Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator. May act as a tumor suppressor in colorectal carcinoma (PubMed:8752209).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Smad2 and Smad3 are intracellular proteins tightly involved in the TGF-β signaling pathway. These proteins also known as mothers against decapentaplegic homolog 2 and 3 play an important role in transmitting signals from the cell surface to the nucleus. Smad2 has a molecular weight of approximately 58 kDa while Smad3 is about 52 kDa. They are expressed in various tissues throughout the body including heart skin and immune cells. Upon activation Smad2 and Smad3 undergo phosphorylation a modification that is important for their function as signal transducers.
Biological function summary	The functions of Smad2 and Smad3 are critical in mediating cellular processes such as proliferation differentiation and apoptosis. These proteins often form complexes specifically oligomeric structures with other Smad proteins like co-Smad4 to exert their regulatory roles. The Smad2/3 complexes control the transcription of specific genes in response to TGF-β stimuli influencing cellular responses in a context-dependent manner.
Pathways	Smad2 and Smad3 are central to the TGF-β signaling pathway one significant pathway that affects cellular growth and differentiation. These proteins interact with TGF-β receptors on the cell surface to become phosphorylated forming a complex with Smad4 which then translocates to the nucleus. In the nucleus this complex regulates gene expression. Both Smad2 and Smad3 also engage with other signaling pathways such as the MAPK pathway further influencing cell behavior and cooperating with proteins like ERK.
Associated diseases and disorders	Smad2 and Smad3 have been linked to conditions like cancer and fibrosis. Dysregulation of Smad2/3 signaling often leads to unchecked cell proliferation a hallmark of tumorigenesis. In fibrosis excessive Smad2/3 activity contributes to tissue scarring due to increased extracellular matrix production. For instance their interaction with proteins like α-SMA (alpha-smooth muscle actin) in fibroblasts plays a role in cardiac and pulmonary fibrosis. Understanding their exact contribution provides insights into potential therapeutic targets for treating these diseases.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

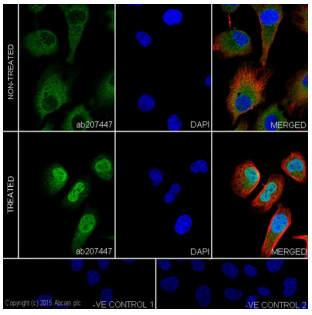
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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10 product images



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

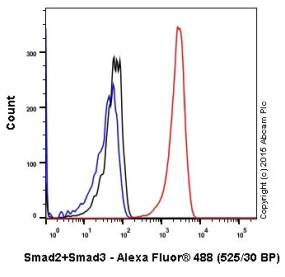
Smad2 + Smad3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad2 + Smad3 antibody

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.

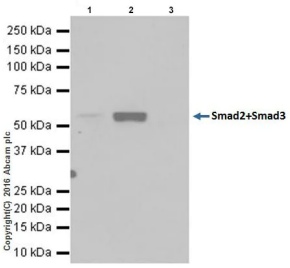
-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary at 1/1000 dilution.



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

Smad2 + Smad3 Flow Cytometry (Intracellular) staining using rabbit Anti-Smad2 + Smad3 antibody

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).

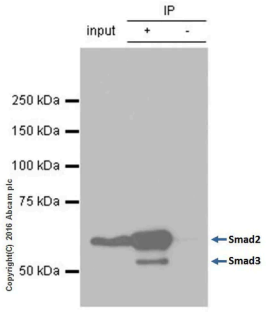
Lane 2: ab207447 IP in HeLa whole cell lysate.

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.

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



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

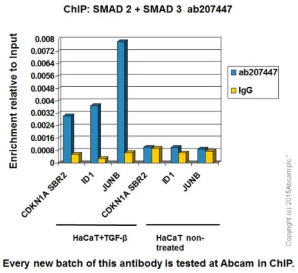
Diluting buffer: 5% NFDM/TBST

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

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

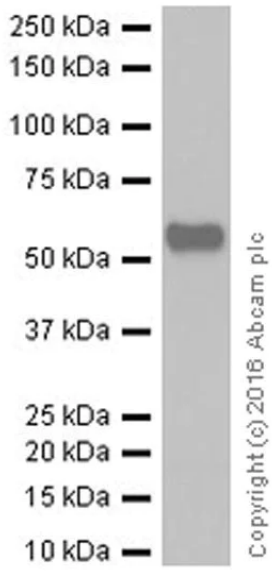
Observed band size: 58-62 kDa

Exposure time: 3min



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.



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

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-Smad2 + Smad3 antibody [EPR19557] - ChIP Grade (ab207447) at 1/2000 dilution

All lanes:
Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate at 10 µg

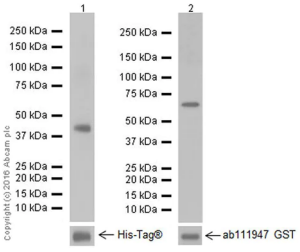
Secondary

All lanes:
Western blot - 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: 15s



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

Smad2 + Smad3 Western blot staining using rabbit Anti-Smad2 + Smad3 antibody

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.

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

Lane 1:
Recombinant protein fragment human Smad2 at 0.01 µg

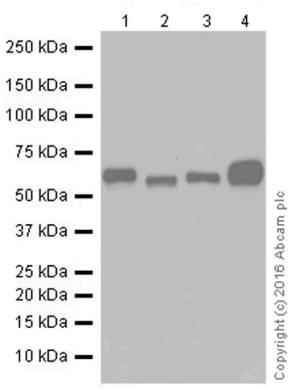
Lane 2:
Recombinant protein fragment human Smad3 at 0.01 µg

Secondary

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

Predicted band size: 52 kDa

Observed band size: 38 kDa, 60 kDa



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

Smad2 + Smad3 Western blot staining using rabbit Anti-Smad2 + Smad3 antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - 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 at 10 µg

Lane 2:
K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate at 10 µg

Lane 3:
A549 (Human lung carcinoma cell line) whole cell lysate at 10 µg

Lane 4:
Human fetal liver lysate at 10 µg

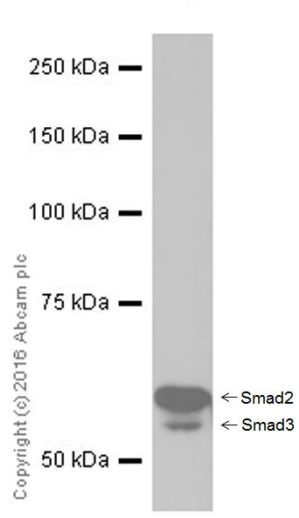
Secondary

All lanes:
Western blot - 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: 3min



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

Blocking/Dilution buffer: 5% NFDM/TBST.
Smad2 and Smad3 can be resolved using a lower percentage gel.

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

All lanes:
HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 10 µg

Secondary

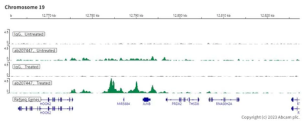
All lanes:

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

Predicted band size: 52 kDa

Observed band size: 52 kDa, 58-62 kDa

Exposure time: 30s



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/mL, 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 found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.