abcam

ab202445

Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade

Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) is a rabbit monoclonal antibody detecting Smad2 + Smad3 in **Western Blot, Flow Cytometry (Intra), IP, ICC/IF, ChIP**. Suitable for **Human, Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 40 publications

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	EPR19557-4
Purification technique	Affinity purification Protein A
Concentration	0.8 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	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/40
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

ChIP

Tested

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

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

WB

Tested

Species	Recombinant fragment - Human
Dilution info	1/1000
Notes	-

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human

Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/200
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/600
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

Target data

See full target information SMAD3

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 SMAD3/SMAD4 complex, activates transcription. Also can form a SMAD3/SMAD4/JUN/FOS complex at the AP-1/SMAD site to regulate TGF-beta-mediated transcription. Has an inhibitory effect on wound healing probably by modulating both growth and migration of primary keratinocytes and by altering the TGF-mediated chemotaxis of monocytes. This effect on wound healing appears to be hormone-sensitive. Regulator of chondrogenesis and osteogenesis and inhibits early healing of bone fractures. Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator.

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

What is this antibody validated in?
Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunocytochemistry/immunofluorescence (ICC/IF), ChIP in Human, Mouse, Rat samples.

What is the molecular weight of Smad2 + Smad3?
Anti-Smad2 + Smad3 [EPR19557-4] - ChIP Grade (ab202445) specifically detects a band for Smad2 + Smad3 (UniProt: Q15796) at a molecular weight of 52kDa.

Trusted by the scientific community
Anti-Smad2 + Smad3 [EPR19557-4] - ChIP Grade (ab202445) was first used in a scientific publication in 2016 and has been cited over 40 times in peer-reviewed journals.

Other related products
We have a range of other formats of antibody clone [EPR19557-4] also available for your convenience: ab202445, Carrier free - ab232326

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.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size 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-B 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-B stimuli influencing cellular responses in a context-dependent manner.
Pathways	Smad2 and Smad3 are central to the TGF-B signaling pathway one significant pathway that affects cellular growth and differentiation. These proteins interact with TGF-B 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

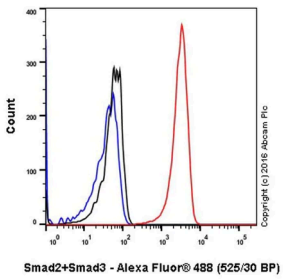
We do not recommend this combination. It is not covered by our product promise.

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.

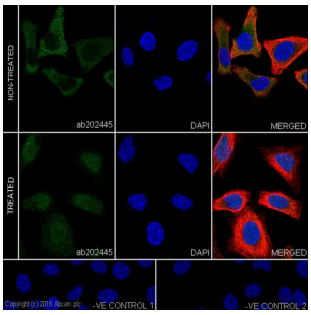
10 product images



Flow Cytometry (Intracellular) - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

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 + Smad3 with ab202445 at 1/600 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 1/2000 dilution was used as the secondary antibody.

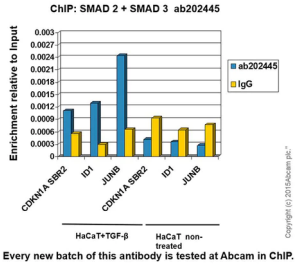


Immunocytochemistry/ Immunofluorescence - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

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

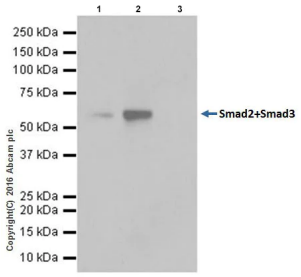
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Smad2 + Smad3 with ab202445 at 1/200 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). The results show signal translocation after TGF-beta (10ng/ml, 1h) treatment on HeLa cells.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: ab202445 at 1/200 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody 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 antibody at 1/1000 dilution.



ChIP - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

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 ab202445 (blue), and 20µl of Anti rabbit 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).



Immunoprecipitation - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

Smad2 + Smad3 was immunoprecipitated from 0.35mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab202445 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab202445 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).

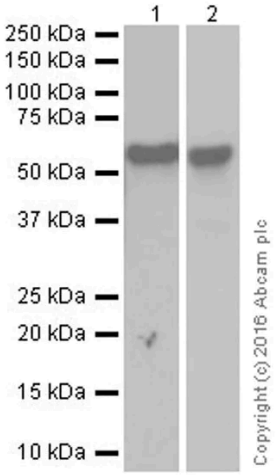
Lane 2: ab202445 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab202445 in HeLa whole cell lysate.

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

Exposure time: 3 seconds.

All lanes:
Immunoprecipitation - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)



Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

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

Blocking/Dilution buffer: 5% NFDM/TBST.
Exposure time: Lane 1: 30 seconds; Lane 2: 10 seconds.

All lanes:
Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) at 1/2000 dilution

Lane 1:
Human fetal heart lysate at 10 µg

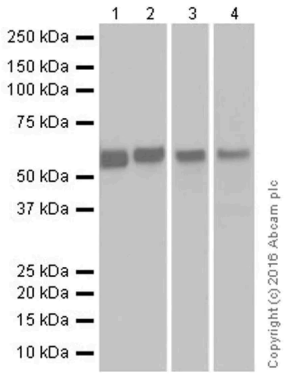
Lane 2:
Human fetal kidney lysate at 10 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 52 kDa

Observed band size: 58-62 kDa



Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

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

Blocking/Dilution buffer: 5% NFDM/TBST.
Exposure time: Lane 1/2: 10 seconds; Lane 3: 3 seconds; Lane 4: 1 seconds.

All lanes:
Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) at 1/2000 dilution

Lane 1:
293 (Human epithelial cell line from embryonic kidney) whole cell lysate at 10 µg

Lane 2:
HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate at 10 µg

Lane 3:
HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 10 µg

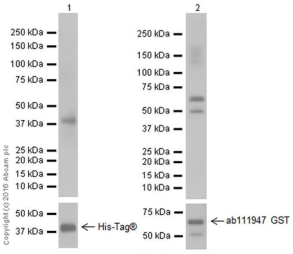
Lane 4:
Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate at 10 µg

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



Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

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

Blocking/Dilution buffer: 5% NFDM/TBST.
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 [EPR19557-4] - ChIP Grade (ab202445) at 1/1000 dilution

Lane 1:
Recombinant protein fragment human Smad2 at 0.01 µg

Lane 2:
Recombinant protein fragment human Smad3 at 0.01 µg

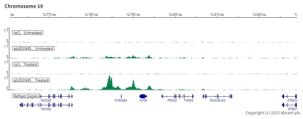
Secondary

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

Predicted band size: 52 kDa

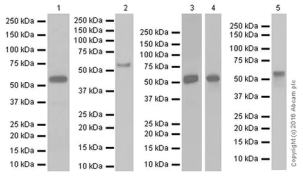
Observed band size: 38 kDa, 60 kDa

Exposure time: 1s



ChIC/CUT&RUN sequencing - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

ChIC/CUT&RUN was performed using ta 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 ab202445 [EPR19557-4]. 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.



Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

Blocking/Dilution buffer: 5% NFDM/TBST.
Exposure time: Lane 1: 3 minutes; Lane 2: 10 seconds; Lane 3: 30 seconds; Lane 4: 3 seconds; Lane 5: 5 seconds.

Lane 1:
Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) at 1/1000 dilution

Lanes 2 and 5:
Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) at 1/2000 dilution

Lanes 3 - 4:
Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) at 1/20000 dilution

Lane 1:
Mouse brain lysate at 10 µg

Lane 2:
Mouse heart lysate at 10 µg

Lane 3:
Rat brain lysate at 10 µg

Lane 4:
C6 (Rat glial tumor cell line) whole cell lysate at 10 µg

Lane 5:
Rat spleen 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

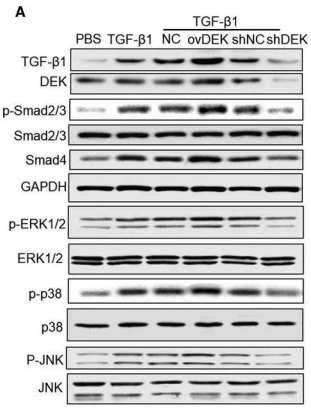


Image collected and cropped by CiteAb under a CC-BY license from the publication

Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

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

Smad2 + Smad3 western blot using anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade ab202445. Publication image and figure legend from Song, Y., Wang, Z., et al., 2020, J Cell Mol Med, PubMed 33124760.

ab202445 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab202445 please see the product overview.

Effects of DEK shRNA on Smad, MAPK and PI3K/AKT signalling pathways in BEAS-2B cells. (A-E) The expressions of TGF-β, DEK mad2/3 and Smad4, ERK1/2, p38, JNK, and PI3K, AKT, mTOR were detected by Western blot. The relative density of each protein was calculated. Data were shown as mean ± SD (n = 3). NC, negative control cells; ovDEK, cells transfected with DEK; shNC, cells transfected with scrambled shRNA; shDEK, cells transfected with DEK shRNA. *p < 0.05, vs control group. #p < 0.05, ovDEK vs TGF-β1 + NC group; shDEK vs TGF-β1 + shNC group

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