abcam

Product datasheet

Anti-Smad3 antibody [EPR19686] - ChIP Grade ab208182

敲除验证 重组 RabMAb

10 References 9 图像

概述

产品名称	Anti-Smad3抗体[EPR19686] - ChIP Grade
描述	兔单克隆抗体[EPR19686] to Smad3 - ChIP Grade
宿主	Rabbit
经测试应用	**适用于:** ChIP, WB, IP
种属反应性	**与反应:** Mouse, Rat, Human, Recombinant fragment
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Human Smad3 recombinant protein; A549, HeLa, Jurkat, HEK-293, K562, BxPC-3, C6, PC-12 and NIH/3T3 whole cell lysates; human fetal kidney lysate; rat spleen and kidney lysates; mouse spleen lysate. IP: HeLa whole cell lysate. ChIP: Chromatin was prepared from HaCaT cells.
常规说明	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**.

性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR19686

同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab208182**于以下的**经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ChIP		Use 2 µg for 25 µg of chromatin.
WB		1/1000. Detects a band of approximately 55 kDa (predicted molecular weight: 48 kDa).
IP		1/30.

靶标

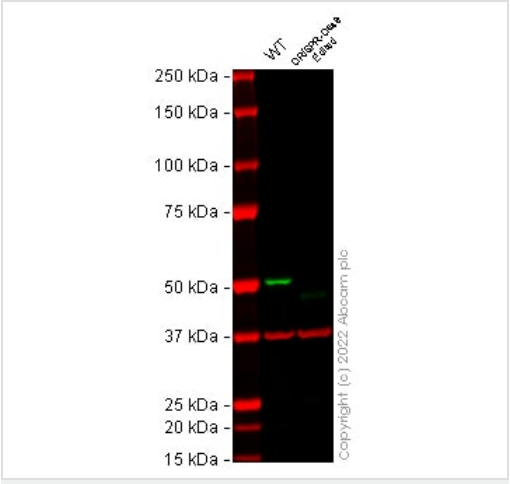
功能	Receptor-regulated SMAD (R-SMAD) that is an intracellular signal transducer and transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinases. Binds the TRE element in the promoter region of many genes that are regulated by TGF-beta and, on formation 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.
疾病相关	Colorectal cancer Loeys-Dietz syndrome 3
序列相似性	Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.
结构域	The MH1 domain is required for DNA binding. Also binds zinc ions which are necessary for the DNA binding. The MH2 domain is required for both homomeric and heteromeric interactions and for transcriptional regulation. Sufficient for nuclear import. The linker region is required for the TGFbeta-mediated transcriptional activity and acts synergistically with the MH2 domain.
翻译后修饰	Phosphorylated on serine and threonine residues. Enhanced phosphorylation in the linker region on Thr-179, Ser-204 and Ser-208 on EGF and TGF-beta treatment. Ser-208 is the main site of MAPK-mediated phosphorylation. CDK-mediated phosphorylation occurs in a cell-cycle dependent manner and inhibits both the transcriptional activity and antiproliferative functions of SMAD3. This phosphorylation is inhibited by flavopiridol. Maximum phosphorylation at the G(1)/S junction. Also phosphorylated on serine residues in the C-terminal SXS motif by TGFBR1 and ACVR1. TGFBR1-mediated phosphorylation at these C-terminal sites is required for interaction with SMAD4, nuclear location and transactivational activity, and appears to be a prerequisite for

the TGF-beta mediated phosphorylation in the linker region. Dephosphorylated in the C-terminal SXS motif by PPM1A. This dephosphorylation disrupts the interaction with SMAD4, promotes nuclear export and terminates TGF-beta-mediated signaling. Phosphorylation at Ser-418 by CSNK1G2/CK1 promotes ligand-dependent ubiquitination and subsequent proteasome degradation, thus inhibiting SMAD3-mediated TGF-beta responses. Phosphorylated by PDPK1. Acetylation in the nucleus by EP300 in the MH2 domain regulates positively its transcriptional activity and is enhanced by TGF-beta.
Ubiquitinated. Monoubiquitinated, leading to prevent DNA-binding. Deubiquitination by USP15 alleviates inhibition and promotes activation of TGF-beta target genes.
Poly-ADP-ribosylated by PARP1 and PARP2. ADP-ribosylation negatively regulates SMAD3 transcriptional responses during the course of TGF-beta signaling.

细胞定位

Cytoplasm. Nucleus. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4 (PubMed:15799969). Through the action of the phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1 (PubMed:16751101, PubMed:19289081). Co-localizes with LEMD3 at the nucleus inner membrane (PubMed:15601644). MAPK-mediated phosphorylation appears to have no effect on nuclear import (PubMed:19218245). PDPK1 prevents its nuclear translocation in response to TGF-beta (PubMed:17327236).

图片



Western blot - Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182)

All lanes : Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : SMAD3 CRISPR-Cas9 edited A549 cell lysate

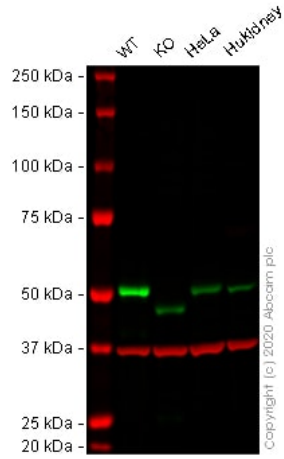
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa
Observed band size: 50 kDa

False colour image of Western blot: Anti-Smad3 antibody [EPR19686] - ChIP Grade staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab208182 was shown to bind specifically to Smad3. A band was observed at 50 kDa in wild-type A549 cell lysates with no signal observed at this size in SMAD3 CRISPR-Cas9 edited cell line **ab277888** (CRISPR-Cas9 edited cell lysate None). The band observed in the CRISPR-Cas9 edited lysate lane below 50 kDa is

likely to represent a truncated form of Smad3. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and SMAD3 CRISPR-Cas9 edited A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182)

All lanes : Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : SMAD3 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : Human Kidney cell lysate

Lysates/proteins at 20 µg per lane.

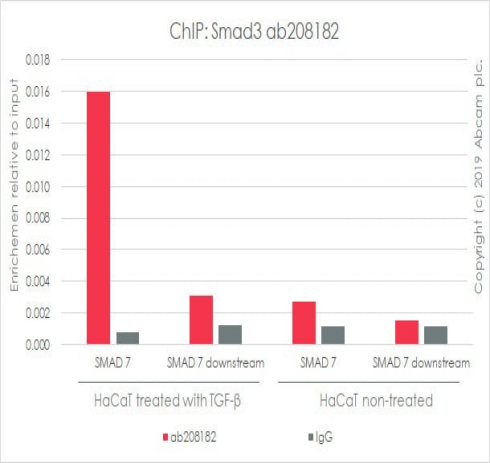
Performed under reducing conditions.

Predicted band size: 48 kDa
Observed band size: 50 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab208182 observed at 50 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab208182 was shown to react with Smad3 in western blot. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab208182 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour

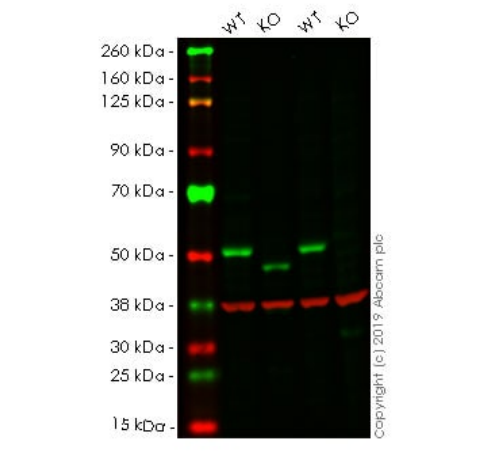
at room temperature before imaging.



ChIP - Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182)

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 ab208182 (red), and 20µl of protein A/G beads. 2µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

The ChIP condition performed here is similar to the literature (PMID: 18245174).



Western blot - Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182)

All lanes : Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : SMAD3 knockout A549 cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : SMAD3 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

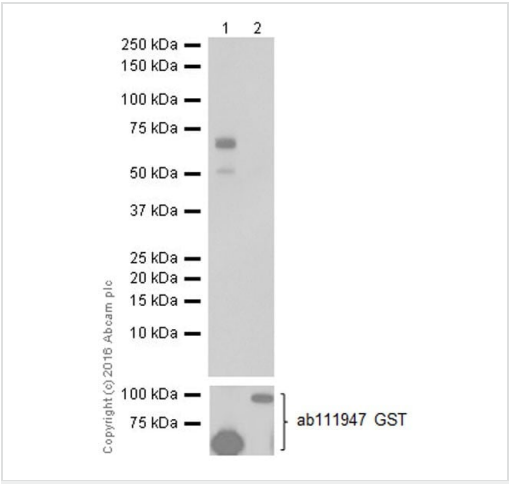
Performed under reducing conditions.

Predicted band size: 48 kDa
Observed band size: 74 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab208182 observed at 55 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab208182 was shown to react with Smad3 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab255431** (knockout cell lysate **ab263834**) was used. Wild-type and Smad3 knockout samples were subjected to SDS-PAGE. ab208182 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit

IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182)

All lanes : Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182) at 1/1000 dilution

Lane 1 : Human Smad3 recombinant protein
Lane 2 : Human Smad2 active protein

Lysates/proteins at 0.01 µg per lane.

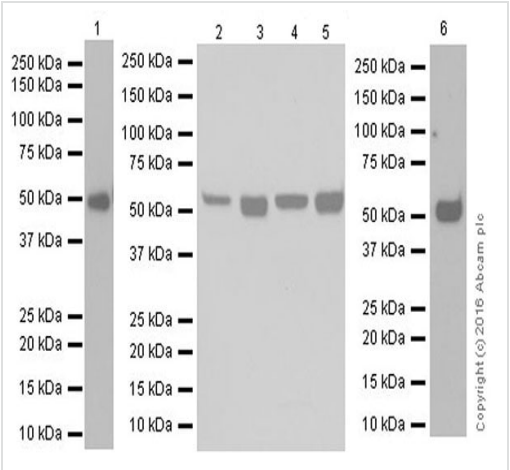
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 48 kDa
Observed band size: 74 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

Human Smad3 recombinant protein contains aa2-227 with GST and His-Tag®. This protein was made-in house.



Western blot - Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182)

All lanes : Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lane 3 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 4 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate
Lane 5 : BxPC-3 (Human pancreas adenocarcinoma cell line) whole cell lysate
Lane 6 : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

Secondary

Lanes 1-5 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

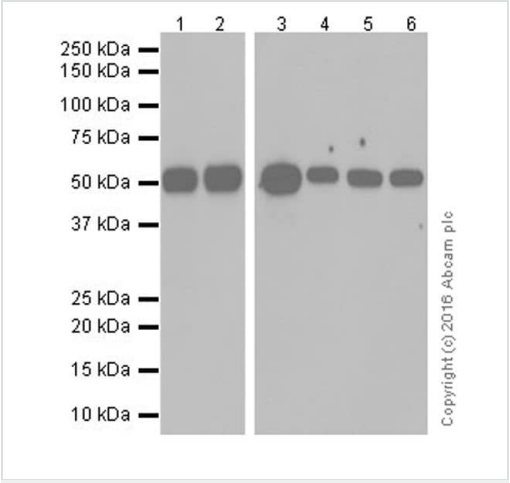
Lane 6 : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 48 kDa

Observed band size: 55 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/6: 3 minutes; Lane 2-5: 15 seconds.



Western blot - Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182)

All lanes : Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182) at 1/1000 dilution

Lane 1 : C6 (Rat glial tumor cell line) whole cell lysate

Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 3 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lane 4 : Rat spleen lysate

Lane 5 : Rat kidney lysate

Lane 6 : Mouse spleen lysate

Lysates/proteins at 10 µg per lane.

Secondary

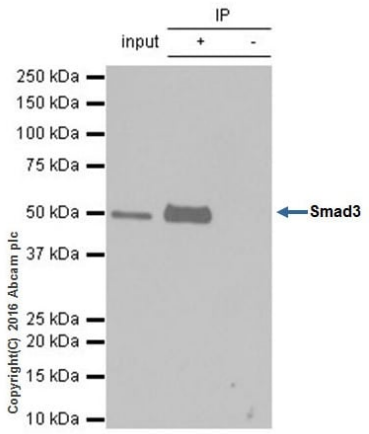
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 48 kDa

Observed band size: 55 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/2: 30 seconds; Lane 3-6: 3 minutes.



Immunoprecipitation - Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182)

Smad3 was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab208182 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab208182 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: ab208182 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab208182 in HeLa whole cell lysate.

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

Exposure time: 3 minutes.

Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182)

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