abcam

Product datasheet

# Anti-Smad3 antibody [EPR19686] - BSA and Azide free ab251490

KO VALIDATED Recombinant RabMAb

5 Images

## Overview

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|---|---|
| **Product name** | Anti-Smad3 antibody [EPR19686] - BSA and Azide free |
| **Description** | Rabbit monoclonal [EPR19686] to Smad3 - BSA and Azide free |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** ChIP, WB, IP |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human, Recombinant fragment |
| **Immunogen** | Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | WB: A549 and HeLa cell lysates; Human kidney cell lysate. ChIP: Chromatin was prepared from HaCaT cells. |
| **General notes** | ab251490 is the carrier-free version of **ab208182**.<br><br>Our **carrier-free** 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.<br><br>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.<br><br>Use our **conjugation kits** 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.<br><br>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.<br><br>This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br><br>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

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| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Store at +4°C. Do Not Freeze. |
| **Storage buffer** | pH: 7.2<br>Constituent: PBS |
| **Carrier free** | Yes |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR19686 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab251490 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 |
|---|---|---|
| **ChIP** | | Use 2 µg for 25 µg of chromatin. |
| **WB** | | Use at an assay dependent concentration. Detects a band of approximately 55 kDa (predicted molecular weight: 48 kDa). |
| **IP** | | Use at an assay dependent concentration. |

## Target

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| **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. |
| **Involvement in disease** | Colorectal cancer<br>Loeys-Dietz syndrome 3 |
| **Sequence similarities** | Belongs to the dwarfin/SMAD family.<br>Contains 1 MH1 (MAD homology 1) domain.<br>Contains 1 MH2 (MAD homology 2) domain. |
| **Domain** | The MH1 domain is required for DNA binding. Also binds zinc ions which are necessary for the |

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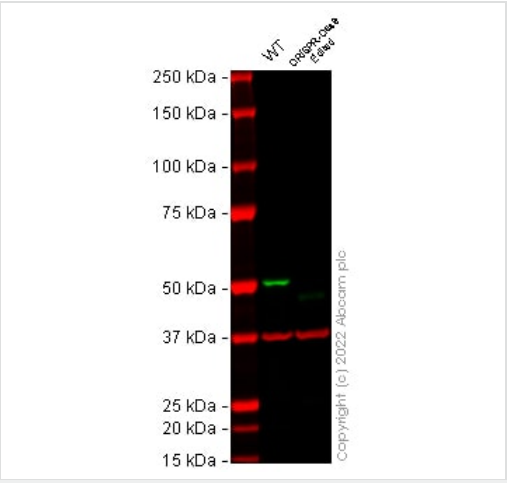
| | DNA binding.<br>The MH2 domain is required for both homomeric and heteromeric interactions and for transcriptional regulation. Sufficient for nuclear import.<br>The linker region is required for the TGFbeta-mediated transcriptional activity and acts synergistically with the MH2 domain. |
| **Post-translational modifications** | 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.<br>Ubiquitinated. Monoubiquitinated, leading to prevent DNA-binding. Deubiquitination by USP15 alleviates inhibition and promotes activation of TGF-beta target genes.<br>Poly-ADP-ribosylated by PARP1 and PARP2. ADP-ribosylation negatively regulates SMAD3 transcriptional responses during the course of TGF-beta signaling. |
| **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 (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). |

## Images




Western blot - Anti-Smad3 antibody [EPR19686] - BSA and Azide free (ab251490)

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

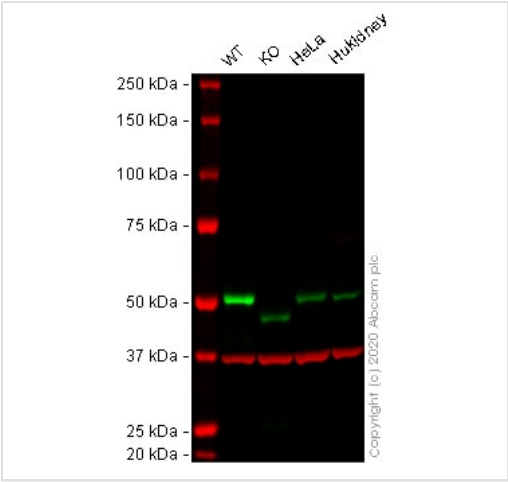
**Lane 1 :** Wild-type A549 cell lysate
**Lane 2 :** SMAD3 CRISPR-Cas9 edited A549 cell lysate

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] - BSA and Azide free (ab251490)

**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.

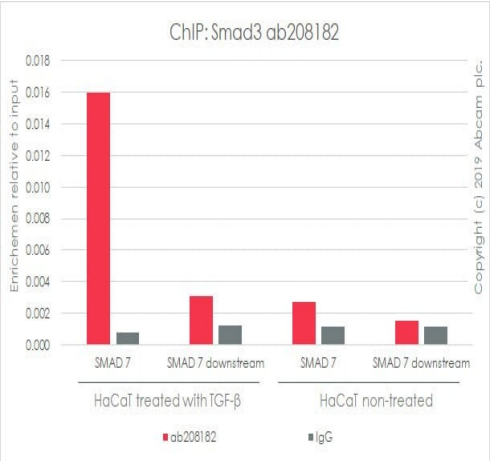
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab208182**).

**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.

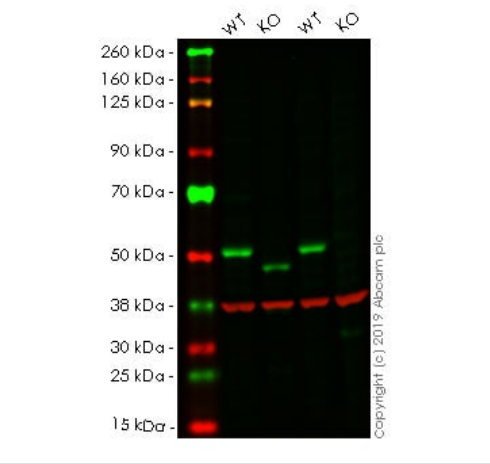



ChIP - Anti-Smad3 antibody [EPR19686] - BSA and Azide free (ab251490)

This data was developed using the same antibody clone in a different buffer formulation (**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] - BSA and Azide free (ab251490)

**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

This data was developed using the same antibody clone in a different buffer formulation (**ab208182**).

**Lanes 1-4:** Merged signal (red and green). Green - **ab208182** observed at 74 kDa. Red - loading control, **ab8245** observed at 37 kDa.

**ab208182** was shown to react with Smad3 in wildtype HeLa. Loss of signal was observed when knockout HeLa cell line **ab255431** (knockout cell lysate **ab263834**). 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.



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