abcam

Product datasheet

Anti-Smad2 + Smad3 antibody [EPR19557] - BSA and Azide free ab236030

Recombinant RabMAb

1 References 6 Images

Overview

Product name	Anti-Smad2 + Smad3 antibody [EPR19557] - BSA and Azide free
Description	Rabbit monoclonal [EPR19557] to Smad2 + Smad3 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIP, IP, ICC/IF, WB
Species reactivity	**Reacts with:** Human, Recombinant fragment
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	ICC/IF: HeLa cells.
General notes	ab236030 is the carrier-free version of **ab207447**. 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. 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. 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. 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. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19557
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab236030 in the following tested applications.

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

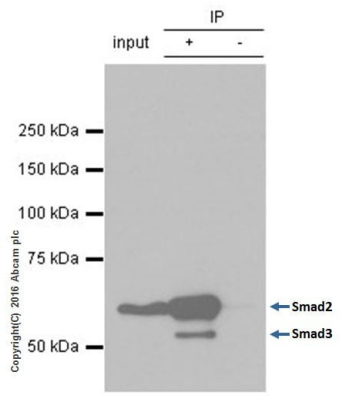
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 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



Immunoprecipitation - Anti-Smad2 + Smad3 antibody [EPR19557] - BSA and Azide free (ab236030)

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.

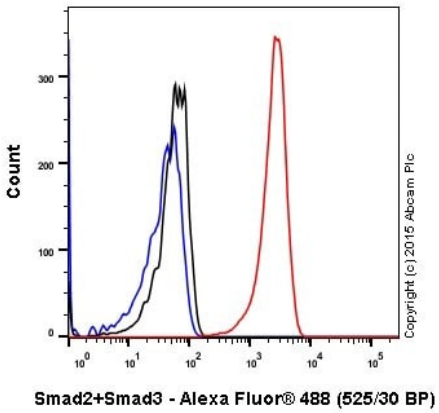
Lane 1: HeLa (human cervix adenocarcinoma) whole cell lysate
Lane 2: HeLa (human cervix adenocarcinoma) whole cell lysate
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab207447** in HeLa (human cervix adenocarcinoma) whole cell lysate

Blocking buffer: 5% NFDM/TBST

Diluting buffer: 5% NFDM/TBST

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

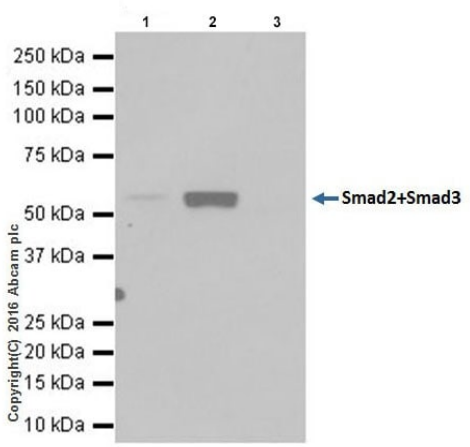
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207447**).



Flow Cytometry (Intracellular) - Anti-Smad2 + Smad3 antibody [EPR19557] - BSA and Azide free (ab236030)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Smad2 and Smad3 with **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 Fluor® 488) at 1/500 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207447**).



Immunoprecipitation - Anti-Smad2 + Smad3 antibody [EPR19557] - BSA and Azide free (ab236030)

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).

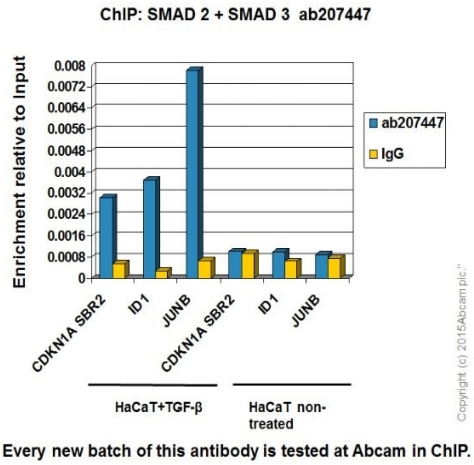
Lane 2: **ab207447** IP in HeLa whole cell lysate.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207447**).

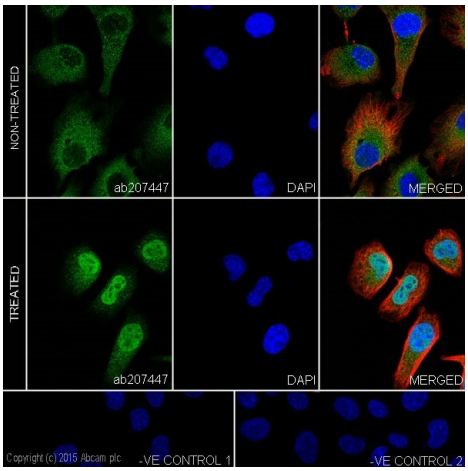


ChIP - Anti-Smad2 + Smad3 antibody [EPR19557] - BSA and Azide free (ab236030)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207447**).



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 + Smad3 antibody [EPR19557] - BSA and Azide free (ab236030)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207447**).

Anti-Smad2 + Smad3 antibody [EPR19557] - BSA and Azide free (ab236030)

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