abcam

ab316118

Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR28610-44
Purification technique	Affinity purification Protein A
Specificity	Not suitable for rat FC and ICC/IF. When tested in dot blot this antibody recognizes Smad2 phosphorylated at both S465 and S467 and does not cross-react with Smad2 when singly phosphorylated at S465 or S467.
Concentration	1.013 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Dot

Tested

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Expected

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

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

IP

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.

Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Not recommended

Species	Rat, Synthetic peptide - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Not recommended

Species	Rat, Synthetic peptide - Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human, Mouse, Rat, Synthetic peptide - Human
Dilution info	-
Notes	-

ChIC/CUT&RUN

Not recommended

Species	Human, Mouse, Rat, Synthetic peptide - Human
Dilution info	-
Notes	-

Target data

See full target information SMAD2 pS465 + S467

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	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C

Notes

ab316118 is the carrirer-free version of ab316117.

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.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

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

Activity summary	Smad2 also known as Mothers against decapentaplegic homolog 2 (MAD2) or MADR2 is a signaling protein involved in the transforming growth factor-beta (TGF-β) receptor pathway. Smad2 has a molecular mass of approximately 58 kDa and expresses in various tissues including epithelial mesenchymal and endothelial cells. Smad2 undergoes phosphorylation on serine residues in response to TGF-β signaling converting it into phosphorylated forms often referred to as p-Smad2 or phospho-Smad2. These phosphorylated forms are critical for the relay of signals from the cell surface to the nucleus.
Biological function summary	Smad2 acts as an intracellular mediator for TGF-β signaling a pathway important for regulating cell proliferation differentiation and apoptosis. Smad2 typically functions as part of a heteromeric complex with Smad4 another key player in TGF-β signaling. Upon activation phosphorylated Smad2 combines with Smad4 to form a complex that translocates into the nucleus. This complex then binds to specific DNA sequences to regulate the transcription of target genes involved in processes such as cell growth inhibition and extracellular matrix production.
Pathways	Smad2 is integral to the TGF-β and activin receptor signaling pathways. These pathways are essential in controlling cell growth and immune responses. Smad2 interacts with other proteins such as Smad3 in addition to Smad4 to modulate gene expression effectively. The interaction between Smad2 and these proteins

ensures precise control of cellular responses to external stimuli emphasizing its pivotal role in maintaining cellular homeostasis.

Associated diseases and disorders

Smad2 correlates with various pathological conditions including fibrosis and cancer. Aberrant Smad2 signaling can contribute to the development of these diseases as excessive TGF-β signaling promotes fibrotic tissue deposition and tumor progression. Smad2 connects with other proteins like Smad3 in these pathological contexts both acting as mediators of abnormal cellular behaviors. Understanding the regulatory mechanisms of Smad2 can help develop therapeutic strategies against disorders linked to dysregulated TGF-β signaling.

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

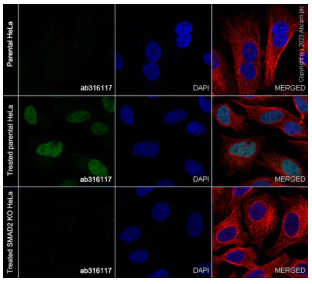
We do not recommend this combination. It is not covered by our product promise.

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.

9 product images



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] - BSA and Azide free (ab316118)

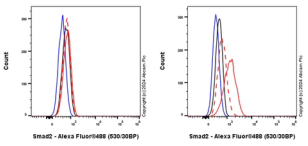
This data was developed using ab316117, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized SMAD2 KO HeLa (human cervical adenocarcinoma epithelial cell) (ab255430) cells labellingSmad2 (phospho S465 + S467) with ab316117 at 1/500 (1.014 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution.

Confocal image showing nuclear and weak cytoplasmic staining in parental HeLa cells (shown in green) treated with TGF-β1 (20 ng/mL) for 1 hr. The counterstain was observed in red. Nuclear DNA was labeled with DAPI (shown in blue). Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/ml) dilution. The Nuclear counterstain was DAPI.

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.

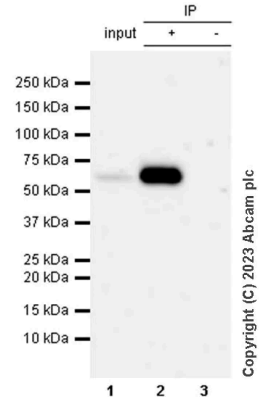


Flow Cytometry (Intracellular) - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] - BSA and Azide free (ab316118)

This data was developed using ab316117, the same antibody clone in a different buffer formulation.

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized (SMAD2 KO HeLa (human cervical adenocarcinoma epithelial cell) treated with 20ng/ml TGF-β1 for 1h (Red) and untreated SMAD2 KO HeLa (dotted red)) (Left) / (Parental HeLa treated with 20ng/ml TGF-β1 for 1h (Red) and untreated HeLa (dotted red)) (Right) cells labelling Smad2 (phospho S465 + S467) with ab316117 at 1/5000 dilution (0.01 ug)/Red and Dotted Red (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] - BSA and Azide free (ab316118)

This data was developed using ab316117, the same antibody clone in a different buffer formulation.

Smad2 (phospho S465 + S467) was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) treated with 20ng/ml TGF beta1 for 60 minutes whole cell lysate with ab316117 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab316117 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) treated with 20ng/ml TGF beta1 for 60 minutes, whole cell lysate
Lane 2: ab316117 IP in HeLa (human cervix adenocarcinoma epithelial cell) treated with 20ng/ml TGF beta1 for 60 minutes whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab316117 in HeLa treated with 20ng/ml TGF beta1 for 60 minutes whole cell lysate

All lanes:
Immunoprecipitation - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] (ab316117) at 1/30 dilution

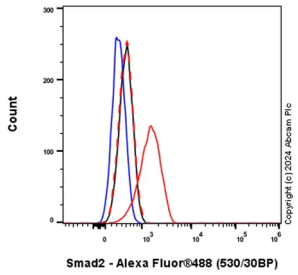
All lanes:

HeLa (human cervix adenocarcinoma epithelial cell) treated with 20ng/ml TGF beta1 for 60 minutes whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 58s

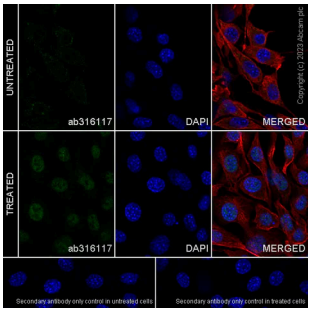


Flow Cytometry (Intracellular) - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] - BSA and Azide free (ab316118)

This data was developed using ab316117, the same antibody clone in a different buffer formulation.

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast) treated with 20ng/ml TGF beta 1 for 15 min (Red) / untreated NIH/3T3 (dotted red) cells labelling Smad2 (phospho S465 + S467) with ab316117 at 1/5000 dilution (0.01 ug)/Red and Dotted Red (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] - BSA and Azide free (ab316118)

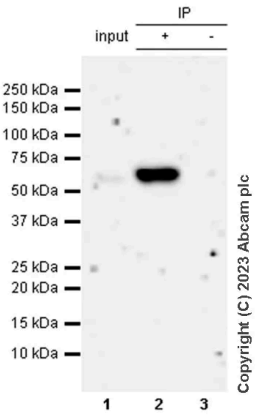
This data was developed using ab316117, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labellingSmad2 (phospho S465 + S467) with ab316117 at 1/500 (1.014 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution.

Confocal image showing nuclear staining in NIH/3T3 cells (shown in green) treated with TGF-β1 (20 ng/mL) for 15 min. The counterstain was observed in red. Nuclear DNA was labeled with DAPI (shown in blue). Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/ml) dilution. The Nuclear counterstain was DAPI.

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.



Immunoprecipitation - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] - BSA and Azide free (ab316118)

This data was developed using ab316117, the same antibody clone in a different buffer formulation.

Smad2 (phospho S465 + S467) was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast) treated with 20ng/ml TGF beta1 for 15 minutes whole cell lysate with ab316117 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab316117 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: NIH/3T3 (mouse embryonic fibroblast) treated with 20ng/ml TGF beta1 for 15 minutes, whole cell lysate
Lane 2: ab316117 IP in NIH/3T3 (mouse embryonic fibroblast) treated with 20ng/ml TGF beta1 for 15 minutes whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab316117 in NIH/3T3 treated with 20ng/ml TGF beta1 for 15 minutes whole cell lysate

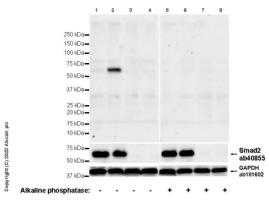
All lanes:
Immunoprecipitation - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] (ab316117) at 1/30 dilution

All lanes:
NIH/3T3 (mouse embryonic fibroblast) treated with 20ng/ml TGF beta1 for 15 minutes whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 180s



Western blot - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] - BSA and Azide free (ab316118)

Smad2 (phospho S465 + S467) Western blot staining using rabbit Anti-Smad2 (phospho S465 + S467) antibody

This data was developed using ab316117, the same antibody clone in a different buffer formulation.

The molecular weight observed is consistent with what has been described in the literature (PMID: 34655614).

The up-regulation of pSmad2 is induced by TGF-beta treatment (PMID: 34655614, 24347165).

In Western blot, ab316117 was shown to bind specifically to Smad2. Target of interest was observed at 60 kDa in wild-type Hela cell lysates (lanes 2) with no signal observed at this size in Smad2 knockout cell line lysates (lanes 3-4 KO cell line ab255430/KO cell lysate ab263833).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-Smad2 antibody [EP784Y] - Total protein control (ab40855) staining at 1/1000 dilution.

All lanes:

Western blot - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] (ab316117) at 1/1000 dilution

Lane 1:
Untreated wild-type HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate (untreated membrane) at 20 µg

Lane 2:
Wild-type Hela treated with 20 ng/ml TGF beta 1 for 60 minutes, whole cell lysate (untreated membrane) at 20 µg

Lane 3:
Untreated Smad2 knockout Hela whole cell lysate (untreated membrane) at 20 µg

Lane 4:
Smad2 knockout Hela treated with 20 ng/ml TGF beta 1 for 60 minutes, whole cell lysate (untreated membrane) at 20 µg

Lane 5:
Untreated wild-type HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

Lane 6:
Wild-type Hela treated with 20 ng/ml TGF beta 1 for 60 minutes, whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

Lane 7:
Untreated Smad2 knockout Hela whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

Lane 8:
Smad2 knockout Hela treated with 20 ng/ml TGF beta 1 for 60 minutes, whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

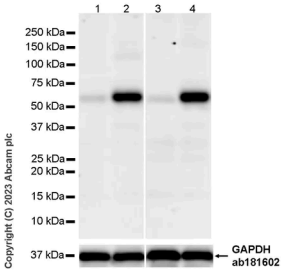
Secondary

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

Performed under reducing conditions.

Observed band size: 60 kDa, 36 kDa

Exposure time: 37s



Western blot - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] - BSA and Azide free (ab316118)

Smad2 (phospho S465 + S467) Western blot staining using rabbit Anti-Smad2 (phospho S465 + S467) antibody

This data was developed using ab316117, the same antibody clone in a different buffer formulation.

The molecular weight observed is consistent with what has been described in the literature (PMID: 34655614).

The up-regulation of pSmad2 is induced by TGF-beta1 treatment (PMID: 34655614, 24347165).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] (ab316117) at 1/1000 dilution

Lane 1:
Untreated NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Lane 2:
NIH/3T3 treated with 20 ng/ml TGF beta 1 for 15 minutes whole cell lysate at 20 µg

Lane 3:
Untreated C6 (rat glial tumor glial cell) whole cell lysate at 20 µg

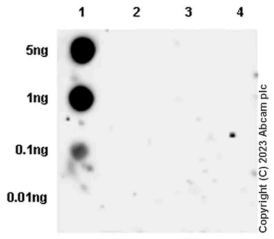
Lane 4:
C6 treated with 10 ng/ml TGF beta 3 for 30 minutes whole cell lysate at 20 µg

Secondary

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

Observed band size: 60 kDa, 36 kDa

Exposure time: 70s



Dot Blot - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] - BSA and Azide free (ab316118)

This data was developed using ab316117, the same antibody clone in a different buffer formulation.

Dot blot analysis of Smad2 (phospho S465 + S467) using ab316117 at 1:1000 (0.507 ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1:100,000 dilution.

The antibody recognizes Smad2 phosphorylated at S465 and S467. It does not cross-react with Smad2 when singly phosphorylated at S465 or S467.

All lanes:
Dot Blot - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] (ab316117) at 1/1000 dilution

Lane 1:
Smad2 (phospho S465+S467) peptide

Lane 2:
Smad2 (phospho S465) peptide

Lane 3:
Smad2 (phospho S467) peptide

Lane 4:
Smad2 non-phospho peptide

Secondary

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

Exposure time: 180s

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