abcam

ab280888

# Anti-Smad2 (phospho S467) antibody [EPR23681-40]

Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888) is a rabbit monoclonal antibody detecting Smad2 in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF, Dot Blot**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 20 publications

Recombinant | RabMAb | KO Validated | 20ul selling size

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | 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** | EPR23681-40 |
| **Purification technique** | Affinity purification Protein A |
| **Specificity** | This antibody was raised against a dual phosphorated peptide for both phosopho S467 and phosopho S465. The dot blot shows that this antibody binds phosopho S467 on its own, but binding is enhanced by the presence of phosopho S465. Our data suggests shown in the dot blot that this antibody does not bind phosopho S465 alone. |
| **Concentration** | 0.519 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

# ICC/IF

## Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/50 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/50 |
| **Notes** | - |

## Expected

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

# IP

## Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/30 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/30 |
| **Notes** | - |

## Expected

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## Dot

### Tested

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | 1/1000 |
| **Notes** | - |

### Expected

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

## WB

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/1000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/1000 |
| **Notes** | - |

### Not recommended

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

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/5000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/5000 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## IHC-P

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/2000 |

| | |
|---|---|
| **Notes** | Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/2000 |
| **Notes** | Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/2000 |
| **Notes** | Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## Target data

See full target information SMAD2 pS467

| | |
|---|---|
| **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** | 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 (phospho S467) antibody [EPR23681-40] (ab280888) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), Dot Blot in Human, Mouse, Rat samples.

**What is the molecular weight of Smad2?**
Anti-Smad2 (phospho S467) [EPR23681-40] (ab280888) specifically detects a band for Smad2 (UniProt: Q15796) at a molecular weight of 52kDa.

**Trusted by the scientific community**
Anti-Smad2 (phospho S467) [EPR23681-40] (ab280888) was first used in a scientific publication in 2021 and has been cited over 20 times in peer-reviewed journals.

**Specificity confirmed**
The specificity of Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888) has been confirmed by Western blot testing in Smad2 Knockout HeLa cell line, ab255430.

**Other related products**
We have a range of other formats of antibody clone [EPR23681-40] also available for your convenience: ab280888, Carrier free - ab280897, APC - ab310849, PE - ab310919, Alexa Fluor® 488 - ab310951, Alexa Fluor® 594 - ab311654, Alexa Fluor® 555 - ab313140

**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 also known as Mothers against decapentaplegic homolog 2 (MAD2) or MADR2 is a signaling protein involved in the transforming growth factor-beta (TGF-B) 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-B 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-B 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-B 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-B 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-B 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-B 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**

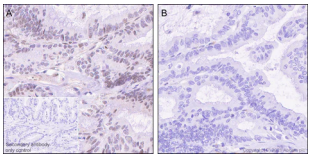
We do not recommend this combination. It is not covered by our product promise.

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.

## 15 product images

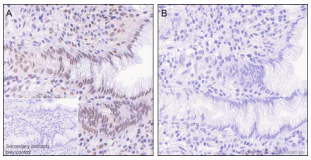


### Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Smad2 (phospho S467) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Smad2 (phospho S467) antibody

Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue labelling Smad2 (phospho S467) with ab280888 at 1/2000 (0.262 µg/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on human colon carcinoma without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab280888 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

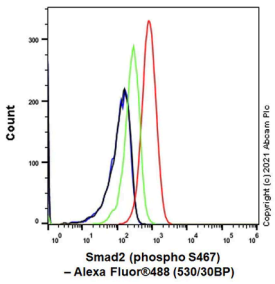


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Smad2 (phospho S467) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Smad2 (phospho S467) antibody

Immunohistochemical analysis of paraffin-embedded human stomach tissue labelling Smad2 (phospho S467) with ab280888 at 1/2000 (0.262 µg/ml) dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on human stomach without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab280888 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

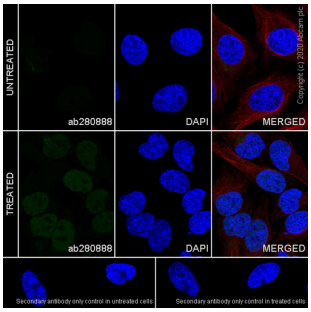



## Flow Cytometry (Intracellular) - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Smad2 (phospho S467) Flow Cytometry (Intracellular) staining using rabbit Anti-Smad2 (phospho S467) antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) treated with 20ng/ml TGF beta1 for 15 minutes (Red)/ Untreated control (Green) cells labelling Smad2 (phospho S467) with ab280888 at 1/5000 dilution (0.01µg)/ 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, ab150077) at 1/2000 dilution was used as the secondary antibody.

The up-regulation of pSmad2 (S467) is induced by TGF-beta1 treatment in HeLa (PMID:24959295).



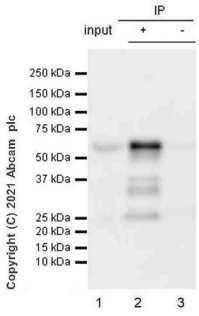

## Immunocytochemistry/ Immunofluorescence - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Smad2 (phospho S467) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad2 (phospho S467) antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labelling Smad2 (phospho S467) with ab280888 at 1/50 (10.48 µg/ml) dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing mainly nuclear staining in HeLa cells treated with TGF-β1 (20 ng/ml) for 15 mins.

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

Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.




## Immunoprecipitation - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Smad2 (phospho S467) was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) treated with 20ng/ml TGF beta1 for 15 minutes, whole cell lysate 10 µg with ab280888 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab280888 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.
**Lane 1**: HeLa treated with 20ng/ml TGF beta1 for 15 minutes, whole cell lysate 10 µg

**Lane 2**: ab280888 IP in HeLa treated with 20ng/ml TGF beta1 for 15 minutes whole cell lysate

**Lane 3**: Rabbit monoclonal IgG (ab172730) instead of ab280888 in HeLa treated with 20ng/ml TGF beta1 for 15 minutes whole cell lysate

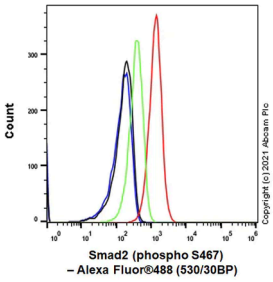
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 15 seconds.

All lanes:
Immunoprecipitation - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Predicted band size: 52 kDa

Observed band size: 60 kDa

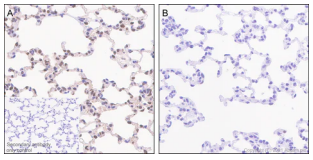



## Flow Cytometry (Intracellular) - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Smad2 (phospho S467) Flow Cytometry (Intracellular) staining using rabbit Anti-Smad2 (phospho S467) antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast) treated with 20ng/ml TGF beta1 for 15 minutes (Red)/ Untreated control (Green) cells labelling Smad2 (phospho S467) with ab280888 at 1/5000 dilution (0.01µg)/ 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, ab150077) at 1/2000 dilution was used as the secondary antibody.

The up-regulation of pSmad2 (S467) is induced by TGF-beta1 treatment in NIH/3T3 (PMID: 24347165).

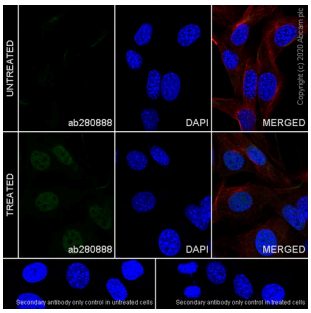


## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Smad2 (phospho S467) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Smad2 (phospho S467) antibody

Immunohistochemical analysis of paraffin-embedded rat lung tissue labelling Smad2 (phospho S467) with ab280888 at 1/2000 (0.262 µg/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on rat lung without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab280888 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



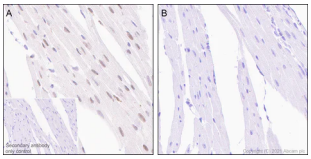

## Immunocytochemistry/ Immunofluorescence - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Smad2 (phospho S467) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad2 (phospho S467) antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 cells labelling Smad2 (phospho S467) with ab280888 at 1/50 (10.48 µg/ml) dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green).
Confocal image showing mainly nuclear staining in NIH/3T3 cells treated with TGF-β1 (20 ng/ml) for 15 mins.

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

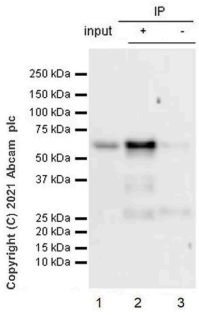
Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.




## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Immunohistochemical analysis of paraffin-embedded mouse cardiac muscle tissue labelling Smad2 (phospho S467) with ab280888 at 1/2000 (0.262 µg/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on mouse cardiac muscle without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab280888 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.




## Immunoprecipitation - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Smad2 (phospho S467) was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast) treated with 20ng/ml TGF beta1 50 µM MG-132 for 15 minutes, whole cell lysate 10 µg with ab280888 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab280888 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.
**Lane 1**: NIH/3T3 treated with 20ng/ml TGF beta1 50 µM MG-132 for 15 minutes, whole cell lysate 10 µg

**Lane 2**: ab280888 IP in NIH/3T3 treated with 20ng/mltreated with 20ng/ml TGF beta1 50 µM MG-132 for 15 minutes, whole cell lysate

**Lane 3**: Rabbit monoclonal IgG (ab172730) instead of ab280888 in NIH/3T3 treated with 20ng/mltreated with 20ng/ml TGF beta1 50 µM MG-132 for 15 minutes, whole cell lysate

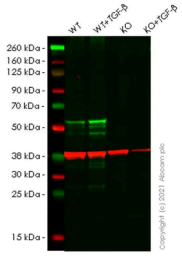
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 32 seconds.

All lanes:
Immunoprecipitation - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Predicted band size: 52 kDa

Observed band size: 60 kDa




## Western blot - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

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

Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS
Lanes 1-4: Merged signal (red and green). Green - ab280888 observed at 60 kDa. Red - loading control ab8245 (Mouse monoclonal [6C5] to GAPDH) observed at 36 kDa.

ab280888 Anti-Smad2 (phospho S467) antibody [EPR23681-40] was shown to specifically react with Smad2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255430 (knockout cell lysate ab263833) was used. Wild-type and Smad2 knockout samples were subjected to SDS-PAGE.

ab280888 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at 4☒ overnight 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 10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888) at 1/1000 dilution

Lane 1:
Wild-type HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Lane 2:
Wild-type HeLa (human cervix adenocarcinoma epithelial cell) treated with 20 ng/ml TGF beta1 for 15 minutes, whole cell lysate at 20 µg

Lane 2:
Western blot - Human SMAD2 knockout HeLa cell line (ab255430)

Lane 3:
Smad2 knockout HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 20 µg

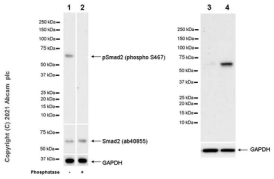
Lane 4:
Smad2 knockout HeLa (human cervix adenocarcinoma epithelial cell), treated with 20 ng/ml TGF beta1 for 15 minutes, whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (ab216773) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (ab216776) at 1/20000 dilution

Predicted band size: 52 kDa

Observed band size: 60 kDa




## Western blot - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

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

Blocking and diluting buffer and concentration: 5% NFDM/TBST
The molecular weight observed is consistent with what has been described in the literature. (PMID: 24959295)

The up-regulation of pSmad2 (S467) is induced by TGF-beta1 treatment in HeLa (PMID:24959295).

Exposure time: 3 minutes

All lanes:
Western blot - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888) at 1/1000 dilution

Lane 1:
HeLa (human cervix adenocarcinoma epithelial cell) treated with 20ng/ml TGF beta1 for 15 minutes, whole cell lysate at 10 µg

Lane 2:
HeLa treated with 20ng/ml TGF beta1 for 15 minutes whole cell lysate (phosphatase treated membrane) at 10 µg

Lane 3:
HeLa whole cell lysate at 20 µg

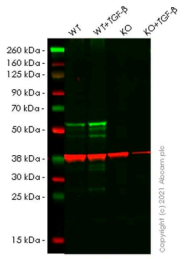
Lane 4:
HeLa treated with 20ng/ml TGF beta1 for 15 minutes, whole cell lysate at 20 µg

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: 60 kDa




## Western blot - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

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

Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS
Lanes 1-4: Merged signal (red and green). Green - ab280888 observed at 60 kDa. Red - loading control ab8245 (Mouse monoclonal [6C5] to GAPDH) observed at 36 kDa.

ab280888 Anti-Smad2 (phospho S467) antibody [EPR23681-40] was shown to specifically react with Smad2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255430 (knockout cell lysate ab263833) was used. Wild-type and Smad2 knockout samples were subjected to SDS-PAGE.

ab280888 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at 4☒ overnight 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 10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888) at 1/1000 dilution

Lane 1:
Wild-type HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Lane 2:
Wild-type HeLa (human cervix adenocarcinoma epithelial cell) treated with 20 ng/ml TGF beta1 for 15 minutes, whole cell lysate at 20 µg

Lane 3:
Smad2 knockout HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 20 µg

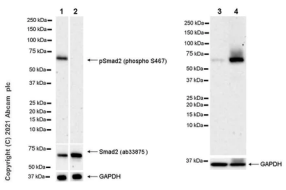
Lane 4:
Smad2 knockout HeLa (human cervix adenocarcinoma epithelial cell), treated with 20 ng/ml TGF beta1 for 15 minutes, whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (ab216773) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (ab216776) at 1/20000 dilution

Predicted band size: 52 kDa

Observed band size: 60 kDa




## Western blot - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Blocking and diluting buffer and concentration: 5% NFDM/TBST
The molecular weight observed is consistent with what has been described in the literature. (PMID: 24347165)

The up-regulation of pSmad2 (S467) is induced by TGF-beta1 treatment in NIH/3T3 (PMID: 24347165)

Exposure time: 3 minutes

All lanes:
Western blot - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888) at 1/1000 dilution

Lane 1:
NIH/3T3 (mouse embryonic fibroblast) treated with 20ng/ml TGF beta1 and 50 µM MG-132 for 15 minutes, whole cell lysate at 10 µg

Lane 2:
NIH/3T3 treated with 20ng/ml TGF beta1 and 50 µM MG-132 for 15 minutes whole cell lysate (phosphatase treated membrane) at 10 µg

Lane 3:
NIH/3T3 whole cell lysate at 10 µg

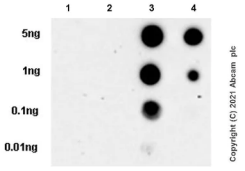
Lane 4:
NIH/3T3 treated with 20ng/ml TGF beta1 and 50 µM MG-132 for 15 minutes, whole cell lysate at 10 µg

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: 60 kDa




## Dot Blot - Anti-Smad2 (phospho S467) antibody [EPR23681-40] (ab280888)

Dot blot analysis of Smad2 (phospho S467) using ab280888 at 1:1000 (0.524 µg/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1:100,000 dilution.
**Lane 1**: Smad2 peptide (aa 458-467)
**Lane 2**: Smad2 (phospho S465) peptide (aa 458-467)
**Lane 3**: Smad2 (phospho aa S465/S467) peptide (aa 458-467)
**Lane 4**: Smad2 (phospho S467) peptide (aa 458-467)

Exposure time: 3 minutes

Blocking and diluting buffer and concentration: 5% NFDM/TBST

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