

Phospho-Smad2-S465/S467 Rabbit mAb

Catalog No.: AP1654 **Recombinant**

Basic Information

Observed MW

60 kDa

Calculated MW

49 kDa/52 kDa

Category

Primary antibody

Applications

WB,IP,IF/ICC,ChIP,ELISA

Cross-Reactivity

Human, Mouse, Rat

CloneNo number

ARC4171

Background

The protein encoded by this gene belongs to the SMAD, 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. This protein mediates the signal of the transforming growth factor (TGF)-beta, and thus regulates multiple cellular processes, such as cell proliferation, apoptosis, and differentiation. This protein is recruited to the TGF-beta receptors through its interaction with the SMAD anchor for receptor activation (SARA) protein. In response to TGF-beta signal, this protein is phosphorylated by the TGF-beta receptors. The phosphorylation induces the dissociation of this protein with SARA and the association with the family member SMAD4. The association with SMAD4 is important for the translocation of this protein into the nucleus, where it binds to target promoters and forms a transcription repressor complex with other cofactors. This protein can also be phosphorylated by activin type 1 receptor kinase, and mediates the signal from the activin. Alternatively spliced transcript variants have been observed for this gene.

Recommended Dilutions

WB 1:4000 - 1:10000**IP** 0.5 µg - 4 µg antibody for
200 µg - 400 µg extracts of
whole cells**IF/ICC** 1:200 - 1:15000**ChIP** 2 µg antibody for 15 µg -
20µg of Chromatin**ELISA** Recommended starting
concentration is 1 µg/mL.
Please optimize the
concentration based on your
specific assay requirements.
For high-ratio antibody
dilutions ($\geq 1:10000$) a
sequential dilution method
is strongly recommended to
ensure measurement
accuracy.

Immunogen Information

Gene ID

4087

Swiss Prot

Q15796

Immunogen

This information is considered to be commercially sensitive.

Synonyms

JV18; LDS6; CHTD8; MADH2; MADR2; JV18-1; hMAD-2; hSMAD2

Product Information

Source

Rabbit

Isotype

IgG

Purification

Affinity purification

Storage

Store at -20°C. Avoid freeze / thaw cycles.

Buffer: PBS with 0.09% Sodium azide, 0.05% BSA, 50% glycerol, pH7.3.

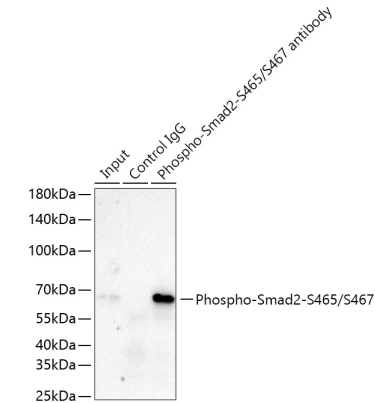
Contact

☎ | 400-999-6126

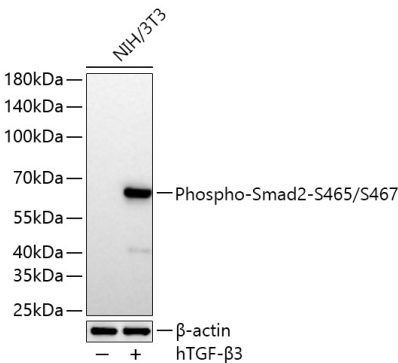
✉ | cn.market@abclonal.com.cn

🌐 | www.abclonal.com.cn

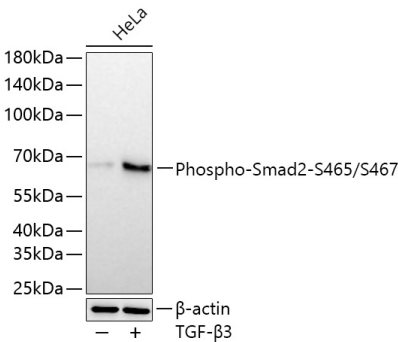
Validation Data



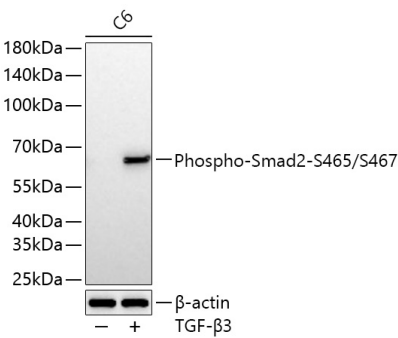
Immunoprecipitation of Phospho-Smad2-S465/S467 from 300 µg extracts of HaCaT cells treated with hTGF-β1 (7 ng/mL, 1 hour) was performed using 0.5 µg of Phospho-Smad2-S465/S467 Rabbit mAb (AP1654). Rabbit Control IgG (AC005) was used to precipitate the Control IgG sample. IP samples were eluted with 1x Laemmli Buffer. The Input lane represents 10% of the total input. Western blot analysis of immunoprecipitates was conducted using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654) at a dilution of 1:10000.



Western blot analysis of lysates from NIH/3T3 cells using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654) at 1:9000 dilution incubated overnight at 4°C. NIH/3T3 cells were treated with TGF-β3 (50 ng/mL) at 37°C for 30 minutes. Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution. Lysates/proteins: 30 µg per lane. Blocking buffer: 3% nonfat dry milk in TBST. Detection: ECL Basic Kit (RM00020). Exposure time: 60 s.

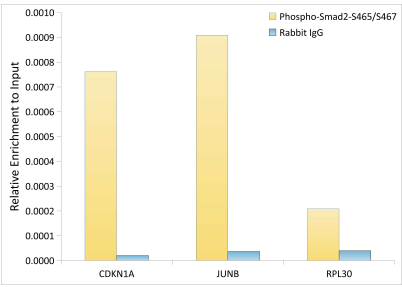


Western blot analysis of lysates from HeLa cells using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654) at 1:9000 dilution incubated overnight at 4°C. HeLa cells were treated with TGF-β3 (50 ng/mL) at 37°C for 30 minutes after serum starvation overnight. Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution. Lysates/proteins: 30 µg per lane. Blocking buffer: 3% nonfat dry milk in TBST. Detection: ECL Basic Kit (RM00020). Exposure time: 90 s.

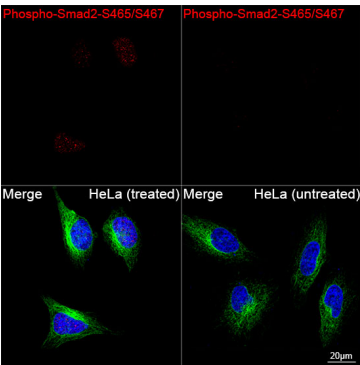


Western blot analysis of lysates from C6 cells using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654) at 1:9000 dilution incubated overnight at 4°C. C6 cells were treated with TGF-β3 (50 ng/mL) at 37°C for 30 minutes. Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution. Lysates/proteins: 30 µg per lane. Blocking buffer: 3% nonfat dry milk in TBST. Detection: ECL Basic Kit (RM00020). Exposure time: 90 s.

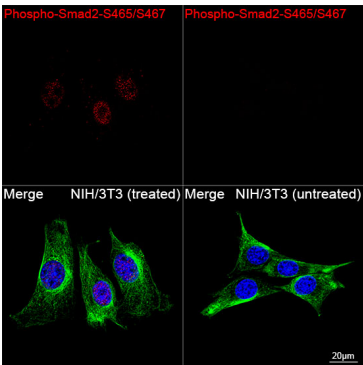
Validation Data



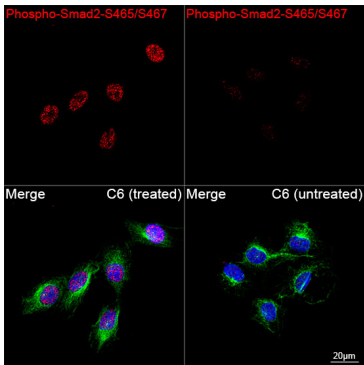
Chromatin immunoprecipitation was performed with 20 µg of cross-linked chromatin from HaCaT cells were treated with hTGF-β1 (7 ng/mL, 1 hour) , using 2 µg of Phospho-Smad2-S465/S467 Rabbit mAb (AP1654) and Rabbit IgG isotype control (AC042). The enrichment of immunoprecipitated DNA at different genomic loci was examined by quantitative PCR. The histogram compares the ratio of the immunoprecipitated DNA to the input at given loci.



Confocal imaging of HeLa cells (treated with TGFβ3) and HeLa cells (untreated) using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654, dilution 1:15000) followed by a further incubation with Cy3-conjugated Goat anti-Rabbit IgG (H+L) (AS007, dilution 1:500) (Red). The cells were counterstained with α-Tubulin Mouse mAb (AC012, dilution 1:400) followed by incubation with ABflo® 488-conjugated Goat Anti-Mouse IgG (H+L) (AS037, dilution 1:800) (Green). DAPI was used for nuclear staining (Blue). Objective: 100x.



Confocal imaging of NIH/3T3 cells (treated with TGFβ3) and NIH/3T3 cells (untreated) using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654, dilution 1:15000) followed by a further incubation with Cy3-conjugated Goat anti-Rabbit IgG (H+L) (AS007, dilution 1:500) (Red). The cells were counterstained with α-Tubulin Mouse mAb (AC012, dilution 1:400) followed by incubation with ABflo® 488-conjugated Goat Anti-Mouse IgG (H+L) (AS037, dilution 1:800) (Green). DAPI was used for nuclear staining (Blue). Objective: 100x.



Confocal imaging of C6 cells (treated with TGFβ3) and C6 cells (untreated) using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654, dilution 1:200) followed by a further incubation with Cy3-conjugated Goat anti-Rabbit IgG (H+L) (AS007, dilution 1:500) (Red). The cells were counterstained with α-Tubulin Mouse mAb (AC012, dilution 1:400) followed by incubation with ABflo® 488-conjugated Goat Anti-Mouse IgG (H+L) (AS037, dilution 1:800) (Green). DAPI was used for nuclear staining (Blue). Objective: 100x.