

# Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467 Rabbit mAb

Catalog No.: AP1518 Recombinant

# **Basic Information**

### **Observed MW**

60kDa

#### **Calculated MW**

52kDa

### Category

Primary antibody

### **Applications**

WB,IHC-P,IF/ICC,ELISA

### **Cross-Reactivity**

Human, Mouse, Rat

#### CloneNo number

ARC59007

# **Background**

The protein encoded by this gene is involved in the transforming growth factor beta signaling pathway that results in an inhibition of the proliferation of hematopoietic progenitor cells. The encoded protein is activated by bone morphogenetic proteins type 1 receptor kinase, and may be involved in cancer. Alternative splicing results in multiple transcript variants.

# **Recommended Dilutions**

**WB** 1:1000 - 1:2000

IHC-P 1:200 - 1:800

**IF/ICC** 1:100 - 1:400

**ELISA** Recommended starting

concentration is 1 µg/mL.

Please optimize the concentration based on your specific assay requirements.

# Immunogen Information

Gene ID Swiss Prot

4086/4090/4093 Q15797/Q99717/O15198

### Immunogen

A synthetic phosphorylated peptide around S463/S465//S467 of human SMAD1/SMAD5/SMAD9.

# **Synonyms**

BSP1; MADH1; MADR1; JV4-1; DWFC; JV5-1; MADH5; MADH6; MADH9; SMAD8; Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467

# **Contact**

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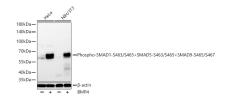
### **Product Information**

SourceIsotypePurificationRabbitIgGAffinity purification

#### Storage

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

Buffer: Buffer: PBS containing 50% glycerol and 0.05% BSA, preserved with proclin300 or sodium azide (as specified on the Certificate of Analysis), pH 7.3.



Western blot analysis of various lysates using Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD5-S465/S467 Rabbit mAb (AP1518) at 1:1000 dilution incubated overnight at 4°C. HeLa cells and NIH/3T3 cells were treated with BMP4 (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: 30s.

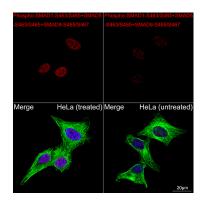


Western blot analysis of lysates from C6 cells using Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467 Rabbit mAb (AP1518) at 1:1000 dilution incubated overnight at 4°C. C6 cells were treated with TGF beta 3 (10 ng/mL) and MG132(2  $\mu$ M) at 37°C for 20 minutes. Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution. Lysates/proteins: 30  $\mu$ g per lane.

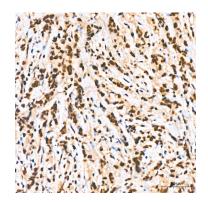
Blocking buffer: 3 % nonfat dry milk in TBST.

Detection: ECL Basic Kit (RM00020).

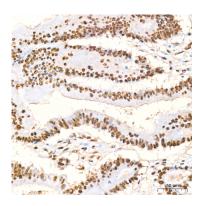
Exposure time: 30s.



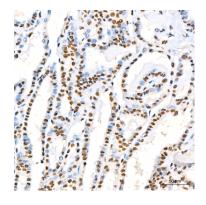
Confocal imaging of HeLa cells (treated with BMP2) and HeLa cells (untreated) using Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467 Rabbit mAb (AP1518, dilution 1:200) followed by a further incubation with Cy3 Goat Anti-Rabbit IgG (H+L) (AS007, dilution 1:500) (Red). The cells were counterstained with  $\alpha$ -tubulin Mouse mAb (AC012, dilution 1:400) followed by incubation with ABflo® 488-conjugated Goat Anti-Mouse IgG (H+L) Ab (AS076, dilution 1:500) (Green). DAPI was used for nuclear staining (Blue). Objective: 100x.



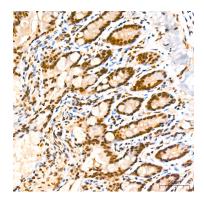
Immunohistochemistry analysis of paraffinembedded Human breast cancer tissue using Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467 Rabbit mAb (AP1518) at a dilution of 1:200 (40x lens). High pressure antigen retrieval performed with 0.01M Citrate Buffer (pH 6.0) prior to IHC staining.



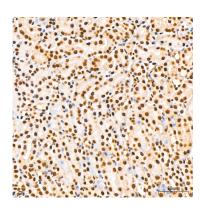
Immunohistochemistry analysis of paraffinembedded Human colon carcinoma tissue using Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467 Rabbit mAb (AP1518) at a dilution of 1:200 (40x lens). High pressure antigen retrieval performed with 0.01M Citrate Buffer (pH 6.0) prior to IHC staining.



Immunohistochemistry analysis of paraffin-

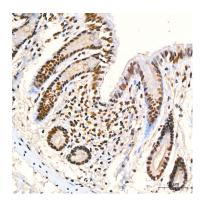


Immunohistochemistry analysis of paraffin-



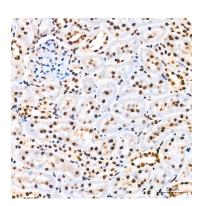
Immunohistochemistry analysis of paraffin-

embedded Human thyroid cancer tissue using Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467 Rabbit mAb (AP1518) at a dilution of 1:200 (40x lens). High pressure antigen retrieval performed with 0.01M Citrate Buffer (pH 6.0) prior to IHC staining.



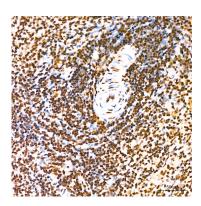
Immunohistochemistry analysis of paraffinembedded Rat colon tissue using Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467 Rabbit mAb (AP1518) at a dilution of 1:200 (40x lens). High pressure antigen retrieval performed with 0.01M Citrate Buffer (pH 6.0) prior to IHC staining.

embedded Moue colon tissue using Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467 Rabbit mAb (AP1518) at a dilution of 1:200 (40x lens). High pressure antigen retrieval performed with 0.01M Citrate Buffer (pH 6.0) prior to IHC staining.



Immunohistochemistry analysis of paraffinembedded Rat kidney tissue using Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467 Rabbit mAb (AP1518) at a dilution of 1:200 (40x lens). High pressure antigen retrieval performed with 0.01M Citrate Buffer (pH 6.0) prior to IHC staining.

embedded Moue kidney tissue using Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467 Rabbit mAb (AP1518) at a dilution of 1:200 (40x lens). High pressure antigen retrieval performed with 0.01M Citrate Buffer (pH 6.0) prior to IHC staining.



Immunohistochemistry analysis of paraffinembedded Rat spleen tissue using Phospho-SMAD1-S463/S465+SMAD5-S463/S465+SMAD9-S465/S467 Rabbit mAb (AP1518) at a dilution of 1:200 (40x lens). High pressure antigen retrieval performed with 0.01M Citrate Buffer (pH 6.0) prior to IHC staining.