Anti-Phospho-Smad3 (Ser423/425) Rabbit mAb

Purified Recombinant Rabbit Monoclonal Antibody

Catalog # R013553

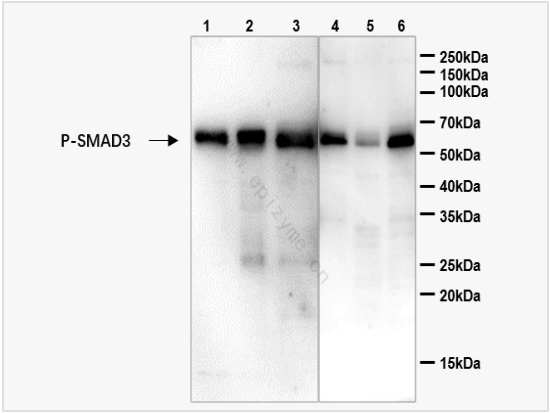
Product Information

Application	IF (Cell)/ICC, WB, ELISA, IHC-P/IF (Tissue-P)
Reactivity	Human, Mouse, Rat
Dilution	WB 1:1,000~1:2,000; IHC-P 1:100~1:200; IF 1:100~1:200
Host	Rabbit
Clonality	Monoclonal
Clone No.	11L61K22
Isotype	IgG
Label	Unconjugated
Immunogen	A synthetic phosphopeptide corresponding to residues surrounding Ser423/Ser425 of human Smad3
Format	Affinity purified monoclonal antibody supplied in PBS with 0.01% sodium azide and 50% glycerol, pH 7.3.
Storage	Shipped on wet ice. Store at -20°C. Stable for 24 months from date of receipt. Aliquoting is unnecessary for -20°C storage.
Precautions	Anti-Phospho-Smad3 (Ser423/425) antibody [11L61K22] is for research use only and not for use in diagnostic or therapeutic procedures.

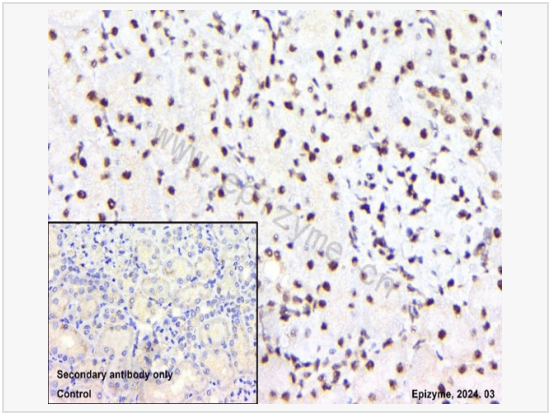
Protein Information

Synonyms	DKFZP586N0721, DKFZp686J10186, hMAD 3, hMAD-3, hSMAD3, HSPC193, HST17436, JV15 2, JV15-2, JV152, LDS1C, LDS3, MAD (mothers against decapentaplegic Drosophila) homolog 3, MAD homolog 3, Mad homolog JV15 2, Mad protein homolog, MAD, mothers against decapentaplegic homolog 3, Mad3, MADH 3, MADH3, MGC60396, Mothers against decapentaplegic homolog 3, Mothers against DPP homolog 3, SMA and MAD related protein 3, SMAD 3, SMAD, SMAD family member 3, SMAD, mothers against DPP homolog 3, Smad3, SMAD3_HUMAN.
Calculated MW	Calculated MW: 55 kDa; Observed MW: 55 kDa
Uniprot ID	P84022
Gene ID	4088
Background	Smad3 transcription factor phosphorylated and activated by TGF-beta-type receptors. A receptor-regulated Smad (R-smad). Binds directly to consensus DNA-binding elements in the promoters of target genes. In mouse required for establishemnt of the mucosal immune response and proper development of skeleton.
Cellular Location	Cytoplasm. Nucleus. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4 (PubMed:15799969). Through the action of the phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1 (PubMed:16751101, PubMed:19289081). Co-localizes with LEMD3 at the nucleus inner membrane (PubMed:15601644). MAPK-mediated phosphorylation appears to have no effect on nuclear import (PubMed:19218245). PDPK1 prevents its nuclear translocation in response to TGF-beta (PubMed:17327236).

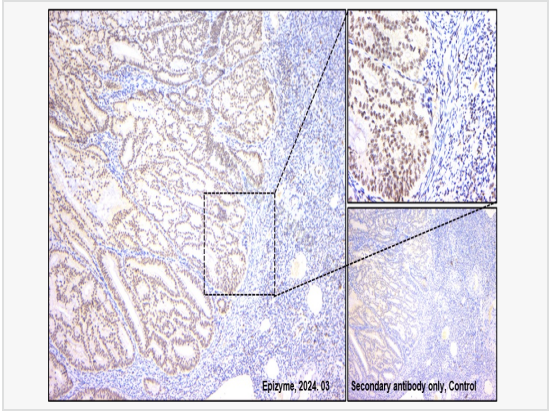
Validation Images



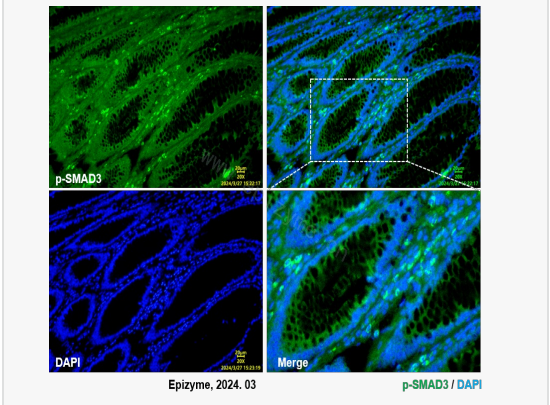
Western Blot - Anti-Phospho-Smad3 (Ser423/425) Rabbit mAb [11L61K22]
All lanes: R013553 at 1:1,000 dilution
Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2: MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysates
Lane 3: A549 (human non-small cell lung cancer epithelial cell) whole cell lysates
Lane 4: SCC-9 (human tongue squamous carcinoma epithelial cell) whole cell lysates
Lane 5: C2C12 (mouse myoblasts epithelial cell) whole cell lysates
Lane 6: U2OS (human osteosarcoma epithelial cell) whole cell lysates
Lysates/proteins at 10 µg per lane.
Secondary antibody: Goat Anti-Rabbit IgG(H+L), HRP Conjugated (Cat. No. LF102) at 1:5,000 dilution
Predicted band size: 55 kDa
Observed band size: 55 kDa
Developed using the ECL technique (Cat. No. SQ201).



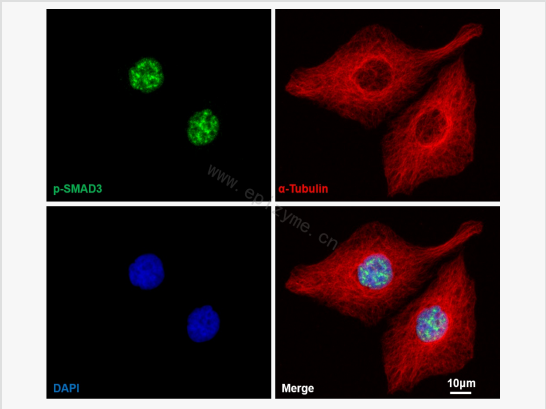
Immunohistochemistry - Anti-Phospho-Smad3 (Ser423/425) Rabbit mAb [11L61K22]
Sample: Paraformaldehyde-fixed, paraffin embedded rat kidney tissue
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins.
Primary antibody: R013553 at 1:200 dilution
Secondary antibody: Goat Anti-Rabbit IgG (H+L), HRP conjugated at 1:1,000 dilution
DAB was used as the chromogen.
Counter stained with hematoxylin.
Positive/negative staining were presented.
Only the secondary antibody was used as the negative control.



Immunohistochemistry - Anti-Phospho-Smad3 (Ser423/425) Rabbit mAb [11L61K22]
Sample: Paraformaldehyde-fixed, paraffin embedded human endometrial cancer tissue
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins.
Primary antibody: R013553 at 1:200 dilution
Secondary antibody: Goat Anti-Rabbit IgG (H+L), HRP conjugated at 1:1,000 dilution
DAB was used as the chromogen.
Counter stained with hematoxylin.
Positive/negative staining were presented.
Only the secondary antibody was used as the negative control.



Immunofluorescence - Anti-Phospho-Smad3 (Ser423/425) Rabbit mAb [11L61K22]
Sample: Paraformaldehyde-fixed, paraffin embedded human colorectal carcinoma tissue
The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins.
Primary antibody: R013553 at 1:100 dilution
Secondary antibody: Goat anti-Rabbit (488) at 1:1,000 dilution (shown in green)
Nuclei were stained with DAPI (shown in blue).



Immunofluorescence - Anti-Phospho-Smad3 (Ser423/425) Rabbit mAb [11L61K22]
Sample: HeLa cells
The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.5% Triton X-100 for 10 minutes and then blocked with 5% BSA in 0.1% PBS-Tween for 0.5 hours.
Primary antibodies: R013553 at 1:100 dilution and α-tubulin Mouse Monoclonal Antibody (Cat. No. LF209) at 1:100 dilution
Secondary antibodies: Goat anti-Rabbit (488) at 1:1,000 dilution (shown in green) and Goat anti-Mouse (CY3) at 1:1,000 dilution (shown in red)
Nuclei were stained with DAPI (shown in blue).



Western Blot - Anti-Phospho-Smad3 (Ser423/425) Rabbit mAb [11L61K22]
All lanes: R013553 at 1:1,000 dilution
Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2: HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates
Lane 3: HCT116 (Human colorectal carcinoma epithelial cell) whole cell lysates
Lysates/proteins at 10 µg per lane.
Secondary antibody: Goat Anti-Rabbit IgG(H+L), HRP Conjugated (Cat. No. LF102) at 1:5,000 dilution
Predicted band size: 55 kDa
Observed band size: 55 kDa
Developed using the ECL technique (Cat. No. SQ201).