



Rabbit Anti-Phospho-Smad3 (Ser423 + Ser425) antibody

SL3425R

Product Name:	Phospho-Smad3 (Ser423 + Ser425)
Chinese Name:	磷酸化细胞Signal transduction分子SMAD3抗体
Alias:	Smad3 (phospho Ser423/425); Smad3 (phospho S423 + S425); p-Smad3 (Ser423 + Ser425); hMAD 3; hSMAD3; HSPC193; JV15 2; JV152; MAD (mothers against decapentaplegic Drosophila) homolog 3; MAD3; MADH 3; MADH3; Mothers against decapentaplegic homolog 3; Mothers against DPP homolog 3; SMA and MAD related protein 3; SMAD 3; SMAD; SMAD-3; SMAD3 HUMAN.
文献引用 	<p>Specific References(4)SL3425R has been referenced in 4 publications.</p> <p>[IF=3.99]Ghiabi, Pegah, et al. "Breast cancer cells promote a notch-dependent mesenchymal phenotype in endothelial cells participating to a pro-tumoral niche." Journal of translational medicine 13.1 (2015): 27.WB;Human. PubMed:25623554</p> <p>[IF=5.29]Shang, Peijin, et al. "Acetyl-11-Keto-β-Boswellic Acid Attenuates Prooxidant and Profibrotic Mechanisms Involving Transforming Growth Factor-β1, and Improves Vascular Remodeling in Spontaneously Hypertensive Rats." Scientific Reports 6 (2016): 39809.WB;Rat. PubMed:28009003</p> <p>[IF=1.84]Bai, Long, et al. "BAMBI promotes porcine granulosa cell steroidogenesis involving TGF-β signaling." Theriogenology (2017).WB;Pig. PubMed:28708530</p> <p>[IF=3.13]Zhang, Wen-feng, et al. "Angelica polysaccharides inhibit the growth and promote the apoptosis of U251 glioma cells in vitro and in vivo." Phytomedicine</p>

	(2017).WB;Human. PubMed:0
Organism Species:	Rabbit
Clonality:	Polyclonal
React Species:	Human,Mouse,Rat,Chicken,Dog,Pig,Cow,Horse,
Applications:	ELISA=1:500-1000IHC-P=1:400-800IHC-F=1:400-800Flow-Cyt=1µg/TestICC=1:100-500IF=1:100-500 (Paraffin sections need antigen repair) not yet tested in other applications. optimal dilutions/concentrations should be determined by the end user.
Molecular weight:	47kDa
Cellular localization:	The nucleuscytoplasmic
Form:	Lyophilized or Liquid
Concentration:	1mg/ml
immunogen:	KLH conjugated Synthesised phosphopeptide derived from human Smad3 around the phosphorylation site of Ser423/425:CS(p-S)V(p-S)
Lsotype:	IgG
Purification:	affinity purified by Protein A
Storage Buffer:	0.01M TBS(pH7.4) with 1% BSA, 0.03% Proclin300 and 50% Glycerol.
Storage:	Store at -20 °C for one year. Avoid repeated freeze/thaw cycles. The lyophilized antibody is stable at room temperature for at least one month and for greater than a year when kept at -20°C. When reconstituted in sterile pH 7.4 0.01M PBS or diluent of antibody the antibody is stable for at least two weeks at 2-4 °C.
PubMed:	PubMed
Product Detail:	<p>Smad3 is a 50 kDa member of a family of proteins that act as key mediators of TGF beta superfamily signaling in cell proliferation, differentiation and development. The Smad family is divided into three subclasses: receptor regulated Smads, activin/TGF beta receptor regulated (Smad2 and 3) or BMP receptor regulated (Smad 1, 5, and 8); the common partner, (Smad4) that functions via its interaction to the various Smads; and the inhibitory Smads, (Smad6 and 7). Activated Smad3 oligomerizes with Smad4 upon TGF beta stimulation and translocates as a complex into the nucleus, allowing its binding to DNA and transcription factors. Phosphorylation of the two TGF beta dependent serines 423 and 425 in the C terminus of Smad3 is critical for Smad3 transcriptional activity and TGF beta signaling.</p> <p>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 SMAD3/SMAD4 complex, activates transcription. Also can form a SMAD3/SMAD4/JUN/FOS complex at the AP-1/SMAD site to regulate TGF-beta-mediated transcription. Has an inhibitory effect on wound healing probably by modulating both growth and migration of primary keratinocytes and by altering the TGF-mediated chemotaxis of monocytes. This effect</p>

on wound healing appears to be hormone-sensitive. Regulator of chondrogenesis and osteogenesis and inhibits early healing of bone fractures (By similarity). Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator.

Subunit:

Monomer; in the absence of TGF-beta. Homooligomer; in the presence of TGF-beta. Heterotrimer; forms a heterotrimer in the presence of TGF-beta consisting of two molecules of C-terminally phosphorylated SMAD2 or SMAD3 and one of SMAD4 to form the transcriptionally active SMAD2/SMAD3-SMAD4 complex. Interacts with TGFBR1. Part of a complex consisting of AIP1, ACVR2A, ACVR1B and SMAD3. Interacts with AIP1, TGFB1I1, TTRAP, FOXL2, PML, PRDM16, HGS and WWP1. Interacts (via MH2 domain) with CITED2 (via C-terminus) (By similarity). Interacts with NEDD4L; the interaction requires TGF-beta stimulation (By similarity). Interacts (via the MH2 domain) with ZFYVE9. Interacts with HDAC1, VDR, TGIF and TGIF2, RUNX3, CREBBP, SKOR1, SKOR2, SNON, ATF2, SMURF2 and TGFB1I1. Interacts with DACH1; the interaction inhibits the TGF-beta signaling. Forms a complex with SMAD2 and TRIM33 upon addition of TGF-beta. Found in a complex with SMAD3, RAN and XPO4. Interacts in the complex directly with XPO4. Interacts (via the MH2 domain) with LEMD3; the interaction represses SMAD3 transcriptional activity through preventing the formation of the heteromeric complex with SMAD4 and translocation to the nucleus. Interacts with RBPMS. Interacts (via MH2 domain) with MECOM. Interacts with WWTR1 (via its coiled-coil domain). Interacts (via the linker region) with EP300 (C-terminal); the interaction promotes SMAD3 acetylation and is enhanced by TGF-beta phosphorylation in the C-terminal of SMAD3. This interaction can be blocked by competitive binding of adenovirus oncoprotein E1A to the same C-terminal site on EP300, which then results in partially inhibited SMAD3/SMAD4 transcriptional activity. Interacts with SKI; the interaction represses SMAD3 transcriptional activity. Component of the multimeric complex SMAD3/SMAD4/JUN/FOS which forms at the AP1 promoter site; required for synergistic transcriptional activity in response to TGF-beta. Interacts (via an N-terminal domain) with JUN (via its basic DNA binding and leucine zipper domains); this interaction is essential for DNA binding and cooperative transcriptional activity in response to TGF-beta. Interacts with PPM1A; the interaction dephosphorylates SMAD3 in the C-terminal SXS motif leading to disruption of the SMAD2/3-SMAD4 complex, nuclear export and termination of TGF-beta signaling. Interacts (dephosphorylated form via the MH1 and MH2 domains) with RANBP3 (via its C-terminal R domain); the interaction results in the export of dephosphorylated SMAD3 out of the nucleus and termination of the TGF-beta signaling. Interacts with MEN1. Interacts with IL1F7. Interaction with CSNK1G2. Interacts with PDPK1 (via PH domain).

Subcellular Location:

Cytoplasm. Nucleus. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4. Through the action of the phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1. Co-localizes with LEMD3 at the

nucleus inner membrane. MAPK-mediated phosphorylation appears to have no effect on nuclear import. PDPK1 prevents its nuclear translocation in response to TGF-beta.

Post-translational modifications:

Phosphorylated on serine and threonine residues. Enhanced phosphorylation in the linker region on Thr-179, Ser-204 and Ser-208 on EGF AND TGF-beta treatment. Ser-208 is the main site of MAPK-mediated phosphorylation. CDK-mediated phosphorylation occurs in a cell-cycle dependent manner and inhibits both the transcriptional activity and antiproliferative functions of SMAD3. This phosphorylation is inhibited by flavopiridol. Maximum phosphorylation at the G(1)/S junction. Also phosphorylated on serine residues in the C-terminal SXS motif by TGFBR1 and ACVR1. TGFBR1-mediated phosphorylation at these C-terminal sites is required for interaction with SMAD4, nuclear location and transactivational activity, and appears to be a prerequisite for the TGF-beta mediated phosphorylation in the linker region. Dephosphorylated in the C-terminal SXS motif by PPM1A. This dephosphorylation disrupts the interaction with SMAD4, promotes nuclear export and terminates TGF-beta-mediated signaling. Phosphorylation at Ser-418 by CSNK1G2/CK1 promotes ligand-dependent ubiquitination and subsequent proteasome degradation, thus inhibiting SMAD3-mediated TGF-beta responses. Phosphorylated by PDPK1. Acetylation in the nucleus by EP300 in the MH2 domain regulates positively its transcriptional activity and is enhanced by TGF-beta. Ubiquitinated.

DISEASE:

Defects in SMAD3 may be a cause of colorectal cancer (CRC) [MIM:114500]. Defects in SMAD3 are the cause of Loeys-Dietz syndrome type 1C (LDS1C) [MIM:613795]. An aortic aneurysm syndrome with widespread systemic involvement. The disorder is characterized by the triad of arterial tortuosity and aneurysms, hypertelorism, and bifid uvula or cleft palate. Patients with LDS1C also manifest early-onset osteoarthritis. They lack craniosynostosis and mental retardation. Note=SMAD3 mutations have been reported to be also associated with thoracic aortic aneurysms and dissection (TAAD) (PubMed:21778426). This phenotype is distinguished from LDS1C by having aneurysms restricted to thoracic aorta. As individuals carrying these mutations also exhibit aneurysms of other arteries, including abdominal aorta, iliac, and/or intracranial arteries (PubMed:21778426), they have been classified as LDS1C by the OMIM resource.

Similarity:

Belongs to the dwarfin/SMAD family.
Contains 1 MH1 (MAD homology 1) domain.
Contains 1 MH2 (MAD homology 2) domain.

SWISS:

P84022

Gene ID:

4088

Database links:

[Entrez Gene: 4088](#) Human

[Entrez Gene: 17127](#) Mouse

[Entrez Gene: 25631](#) Rat

[Omid: 603109](#) Human

[SwissProt: P84022](#) Human

[SwissProt: Q8BUN5](#) Mouse

[SwissProt: P84025](#) Rat

[Unigene: 727986](#) Human

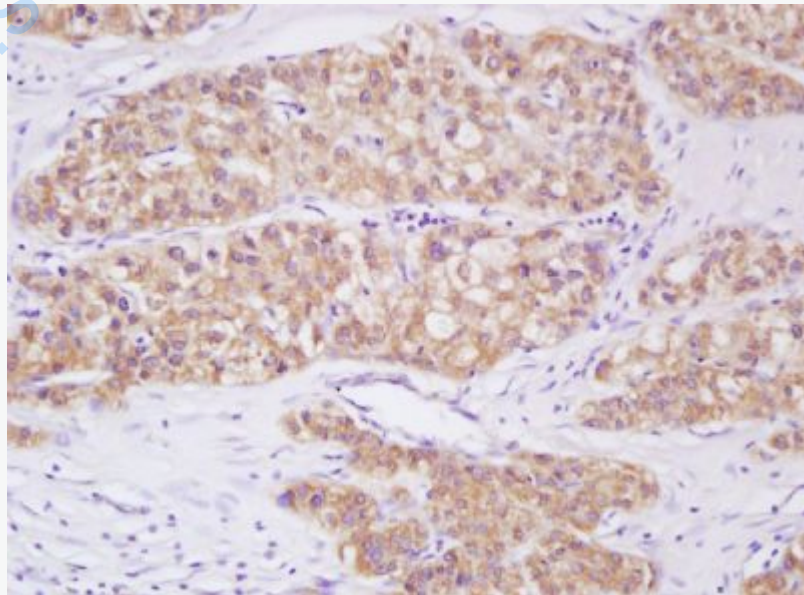
[Unigene: 7320](#) Mouse

[Unigene: 10636](#) Rat

Important Note:

This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.

Picture:

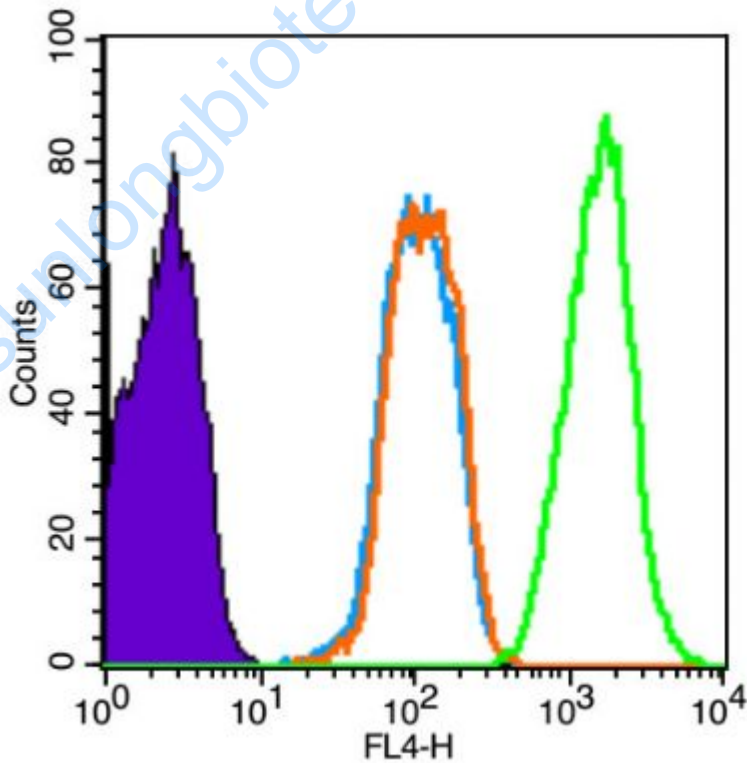


Tissue/cell: human liver carcinoma; 4% Paraformaldehyde-fixed and paraffin-embedded;

Antigen retrieval: citrate buffer (0.01M, pH 6.0), Boiling bathing for 15min; Block endogenous peroxidase by 3% Hydrogen peroxide for 30min; Blocking buffer (normal goat serum,C-0005) at 37°C for 20 min;

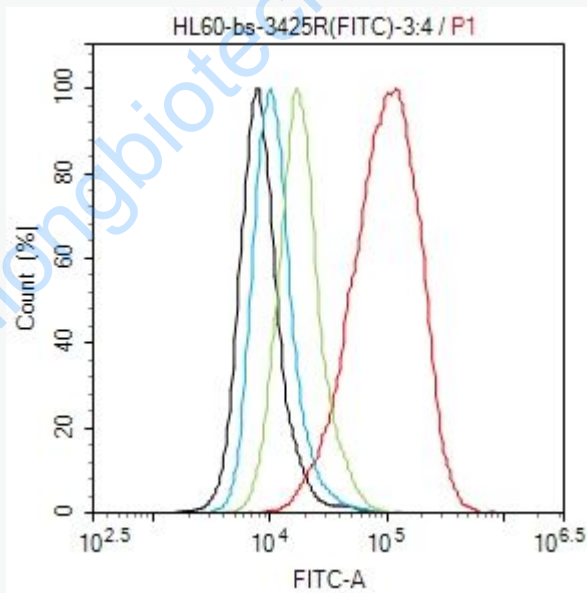
Incubation: Anti-Phospho-Smad3(Ser423/425) Polyclonal Antibody,

Unconjugated(SL3425R) 1:200, overnight at 4°C, followed by conjugation to the secondary antibody(SP-0023) and DAB(C-0010) staining



Blank control (Black line): HUVEC (Black). Primary Antibody (green line): Rabbit Anti-Phospho-Smad3 (Ser423 + Ser425) antibody (SL3425R) Dilution: $1\mu\text{g}/10^6$

cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody (white blue line): Goat anti-rabbit IgG-AF647 Dilution: 1 μ g /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 90% ice-cold methanol for 20 min at room temperature. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.

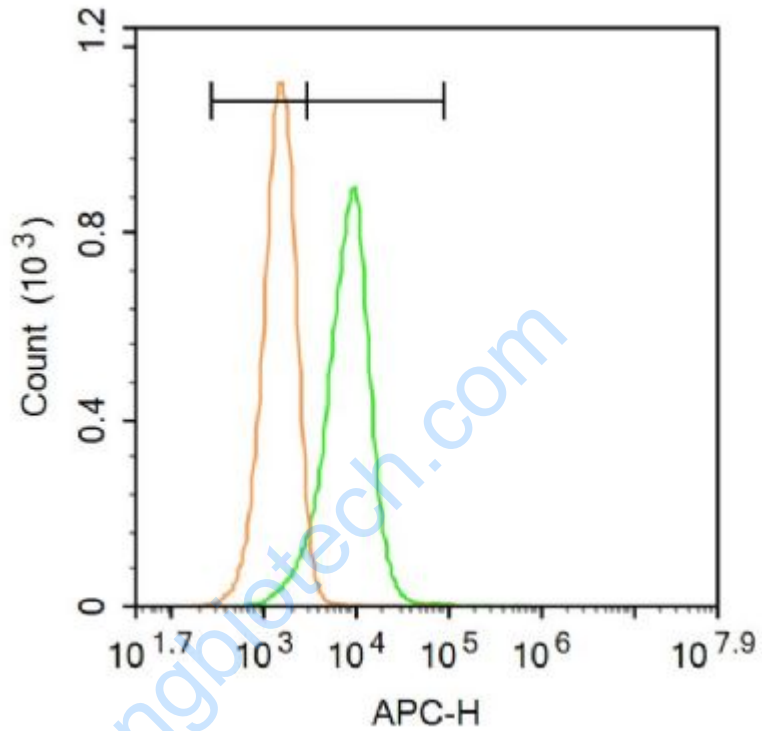


Blank control (black line): HL60(black) (The cells were fixed with 2% paraformaldehyde (10 min) , then permeabilized with PBST for 30 min on room temperature)

Primary Antibody (Red line): Rabbit Anti-Phospho-Smad3(Ser423+Ser425) antibody (SL3425R) ; Dilution: 1 μ g /10⁶ cells;

Isotype Control Antibody (green line): Rabbit IgG .

Secondary Antibody (red line): Goat anti-rabbit IgG-FITC; Dilution: 1 μg /test.



Blank control: HUVEC (Black).

Primary Antibody (green line): Rabbit Anti-Phospho-Jak1 antibody (SL3425R)

Dilution: 1 μg /10⁶ cells;

Isotype Control Antibody (orange line): Rabbit IgG .

Secondary Antibody: Goat anti-rabbit IgG-AF647

Dilution: 1 μg /test.

Protocol

The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room

	temperature. Acquisition of 20,000 events was performed.
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