## Phospho-Smad3 (Ser425) Ab

Cat.#: AF3362 Concn.: 1mg/ml Mol.Wt.: 58kDa Size: 100ul,200ul Source: Rabbit Clonality: Polyclonal

Application: WB 1:500-1:2000 IHC 1:50-1:200, IF/ICC 1:100-1:500

Reactivity: Human, Mouse, Rat

Purification: The Ab is from purified rabbit serum by affinity purification

via sequential chromatography on phospho- and non-

phospho-peptide affinity columns.

Specificity: Phospho-Smad3 (Ser425) Ab detects endogenous levels of

Smad3 only when phosphorylated at Serine 425.

Immunogen: A synthesized peptide derived from human Smad3 around

the phosphorylation site of Serine 425.

Uniprot: P84022

Description: 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.

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 (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).

Similarity: The MH1 domain is required for DNA binding. Also binds zinc

ions which are necessary for the DNA binding. The MH2 domain is required for both homomeric and heteromeric interactions and for transcriptional regulation. Sufficient for nuclear import. The linker region is required for the TGF betamediated transcriptional activity and acts synergistically with the MH2 domain. Belongs to the dwarfin/SMAD family.

Storage Condition and

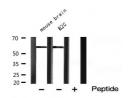
Buffer:

Rabbit IgG in phosphate buffered saline , pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol. Store at -20

°C.Stable for 12 months from date of receipt.



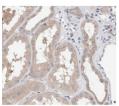
## Affinity Biosciences website:www.affbiotech.com order:order@affbiotech.com



Western blot analysis of extracts of various tissue sample, Phospho-Smad3 (Ser425) Ab.



Western blot analysis of Smad3 phosphorylation expression in NIH-3T3 whole cell lysates, The lane on the left is treated with the antigen-specific peptide.



AF3362 at 1/200 staining human kidney tissue sections by IHC-P. The tissue was formaldehyde fixed and a heat mediated antigen retrieval step in citrate buffer was performed. The tissue was then blocked and incubated with the Ab for 1.5 hours at 22°C. An HRP conjugated goat anti-rabbit Ab was used as the secondary.



AF3362 staining NIH-3T3 by IF/ICC. The sample were fixed with PFA and permeabilized in 0.1% Triton X-100,then blocked in 10% serum for 45 minutes at 25°C. The primary Ab was diluted at 1/200 and incubated with the sample for 1 hour at 37°C. An Alexa Fluor 594 conjugated goat anti-rabbit IgG (H+L) Ab, diluted at 1/600, was used as the secondary Ab.

<code>IMPORTANT:</code> For western blot, incubate membrane with diluted primary Ab in 5% w/v milk , 1% TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

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