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# Smad3 (Phospho Ser425) Rabbit pAb

CatalogNo: YP0585 Orthogonal Validated 💽

# Key Features

| Host Species <ul> <li>Rabbit</li> </ul> | Reactivity <ul> <li>Human,Mouse,Rat</li> </ul> | Applications<br>• IF,WB,IHC,ELISA |
|---|--|-----------------------------------|
| MW<br>• 58kD (Observed)                 | Isotype<br>• IgG                               |                                   |

### **Recommended Dilution Ratios**

IF 1:50-200 WB 1:500-1:2000 IHC 1:100-1:300 ELISA 1:40000 Not yet tested in other applications

### **Storage**

| Storage*    | -15°C to -25°C/1 year(Do not lower than -25°C)                          |
|-------------|---|
| Formulation | Liquid in PBS containing 50% glycerol, 0.5% BSA and 0.02% sodium azide. |

#### **Basic Information**

Clonality Polyclonal

# Immunogen Information

ImmunogenThe antiserum was produced against synthesized peptide derived from human Smad3<br/>around the phosphorylation site of Ser425. AA range:376-425

Specificity

Phospho-Smad3 (S425) Polyclonal Antibody detects endogenous levels of Smad3 protein only when phosphorylated at S425.The name of modified sites may be influenced by many factors, such as species (the modified site was not originally found in human samples) and the change of protein sequence (the previous protein sequence is incomplete, and the protein sequence may be prolonged with the development of protein sequencing technology). When naming, we will use the "numbers" in historical reference to keep the sites consistent with the reports. The antibody binds to the following modification sequence (lowercase letters are modification sites):CSSVs

## Target Information

Gene name SMAD3 MADH3

#### Protein Name Mothers against decapentaplegic homolog 3

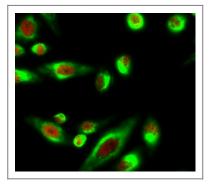
| Organism | Gene ID       | UniProt ID     |
|----------|---------------|----------------|
| Human    | <u>4088;</u>  | <u>P84022;</u> |
| Mouse    | <u>17127;</u> | <u>Q8BUN5;</u> |
| Rat      | <u>25631;</u> | <u>P84025;</u> |

Cellular Localization
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, PubMed:21145499). 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). Localized mainly to the nucleus in the early stages of embryo development with expression becoming evident in the cytoplasm of the inner cell mass at the blastocyst stage (By similarity). .

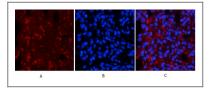
**Tissue specificity** Brain, Colon carcinoma, Esophagus tumor, Pancreas, Placenta, Spleen, Umbilical cord blood

Function Disease:Defects in SMAD3 may be a cause of colorectal cancer (CRC) [MIM:114500]., Domain: The MH2 domain is sufficient to carry protein nuclear export., Function: Transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinase. SMAD3 is a receptor-regulated SMAD (R-SMAD)., PTM: Phosphorylated on serine by TGF-beta and activin type 1 receptor kinases., similarity: Belongs to the dwarfin/SMAD family., similarity: Contains 1 MH1 (MAD homology 1) domain., similarity: Contains 1 MH2 (MAD homology 2) domain., subcellular location: In the cytoplasm in the absence of ligand. Migration to the nucleus when complexed with Smad4., subunit: Interacts with HGS. Interacts with NEDD4L in response to TGF-beta. Interacts with TTRAP (By similarity). Interacts with SARA (SMAD anchor for receptor activation); form trimers with another SMAD3 and the co-SMAD SMAD4. Interacts with IUN/FOS, vitamin D receptor, homeobox protein TGIF and TGIF2, PEBP2-alpha C subunit, CREB-binding protein (CBP), p300, SKI, SNON, ATF2, SMURF2, AIP1, DACH1 and TGFB1I1. Part of a complex consisting of AIP1, ACVR2A, ACVR1B and SMAD3. Found in a complex with SMAD2 and TRIM33 upon addition of TGF-beta. Interacts with SMAD2 and TRIM33. Found in a complex with SMAD3, Ran and XPO4. Interacts with XPO4. Interacts with LBXCOR1 and CORL2.,

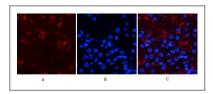
# Validation Data



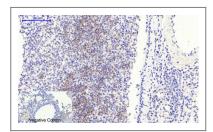
Immunofluorescence analysis of Hela cell. 1,Smad3 (phospho Ser425) Polyclonal Antibody(red) was diluted at 1:200(4° overnight). ATG5 mouse Monoclonal Antibody(3C7)(green) was diluted at 1:200(4° overnight). 2, Goat Anti Rabbit Alexa Fluor 594 Catalog:RS3611 was diluted at 1:1000(room temperature, 50min). Goat Anti Mouse Alexa Fluor 488 Catalog:RS3208 was diluted at 1:1000(room temperature, 50min).



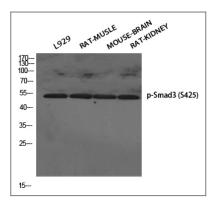
Immunofluorescence analysis of rat-lung tissue. 1,Smad3 (phospho Ser425) Polyclonal Antibody(red) was diluted at 1:200(4°C,overnight). 2, Cy3 labled Secondary antibody was diluted at 1:300(room temperature, 50min).3, Picture B: DAPI(blue) 10min. Picture A:Target. Picture B: DAPI. Picture C: merge of A+B



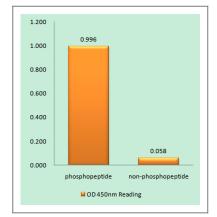
Immunofluorescence analysis of mouse-kidney tissue. 1,Smad3 (phospho Ser425) Polyclonal Antibody(red) was diluted at 1:200(4°C,overnight). 2, Cy3 labled Secondary antibody was diluted at 1:300(room temperature, 50min).3, Picture B: DAPI(blue) 10min. Picture A:Target. Picture B: DAPI. Picture C: merge of A+B



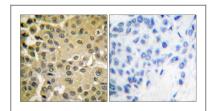
Immunohistochemical analysis of paraffin-embedded Mouse-lung tissue. 1,Smad3 (phospho Ser425) Polyclonal Antibody was diluted at 1:200(4°C,overnight). 2, Sodium citrate pH 6.0 was used for antibody retrieval(>98°C,20min). 3,Secondary antibody was diluted at 1:200(room tempeRature, 30min). Negative control was used by secondary antibody only.



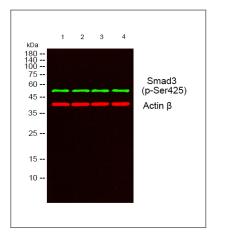
Western Blot analysis of various cells using Phospho-Smad3 (S425) Polyclonal Antibody diluted at 1:500



Enzyme-Linked Immunosorbent Assay (Phospho-ELISA) for Immunogen Phosphopeptide (Phospho-left) and Non-Phosphopeptide (Phospho-right), using Smad3 (Phospho-Ser425) Antibody



Immunohistochemistry analysis of paraffin-embedded human breast carcinoma, using Smad3 (Phospho-Ser425) Antibody. The picture on the right is blocked with the phospho peptide.



Western blot analysis of lysates from 1) L929, 2) RAT MUSLE,3)MOUSE-BRAIN, 4) RAT- KIDNEY cells, (Green) primary antibody was diluted at 1:1000, 4°over night, secondary antibody(cat:RS23920)was diluted at 1:10000, 37° 1hour. (Red) Actin  $\beta$  Monoclonal Antibody(5B7) (cat:YM3028) antibody was diluted at 1:5000 as loading control, 4° over night,secondary antibody(cat:RS23710)was diluted at 1:10000, 37° 1hour.

### **Contact information**

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