

## Anti-SMAD3 Antibody (8P334)

## Product Details

Ig Type:	IgG
Reactivity:	Human,Mouse,Rat
Molecular Weight:	Theoretical: 47 kDa. Actual: 50 kDa.
Clone:	8P334
Purification:	Protein A purified

## Applications

1. HT-1080 cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (Smad3) monoclonal Antibody, Unconjugated (TMAB-01740) 1:100, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nucleus.

2. Blank control: HUVEC.

Primary Antibody (green line): Rabbit Anti-Smad3 antibody (TMAB-01740)

Dilution: 1 µg/Test;

Secondary Antibody: Goat anti-rabbit IgG-FITC

Dilution: 0.5 µg/Test.

Protocol

The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with 0.1% PBST 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.

3. Paraformaldehyde-fixed, paraffin embedded Mouse Liver; Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Antibody incubation with Smad3 Monoclonal Antibody, Unconjugated (TMAB-01740) at 1:200 overnight at 4°C, followed by conjugation to the secondary antibody and DAB staining.

4. Paraformaldehyde-fixed, paraffin embedded Rat Liver; Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Antibody incubation with Smad3 Monoclonal Antibody, Unconjugated (TMAB-01740) at 1:200 overnight at 4°C, followed by conjugation to the secondary antibody and DAB staining.

5. Paraformaldehyde-fixed, paraffin embedded Human Liver; Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Antibody incubation with Smad3 Monoclonal Antibody, Unconjugated (TMAB-01740) at 1:200 overnight at 4°C, followed by conjugation to the secondary antibody and DAB staining.

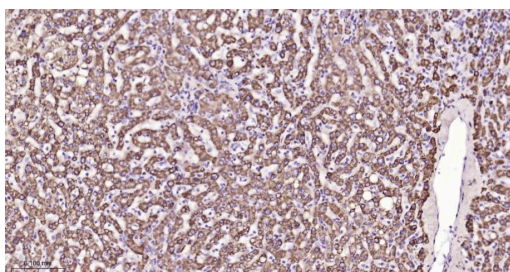
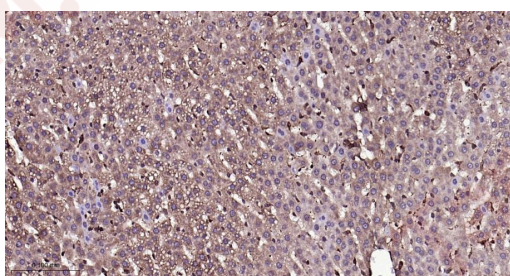
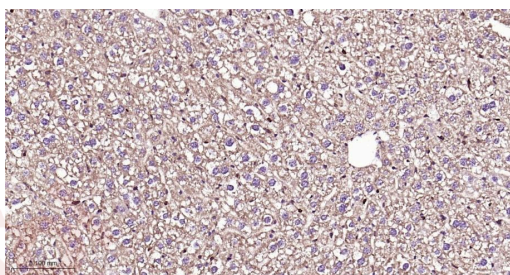
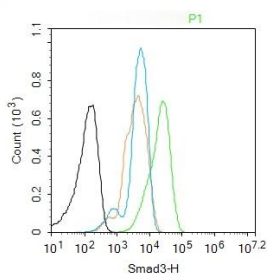
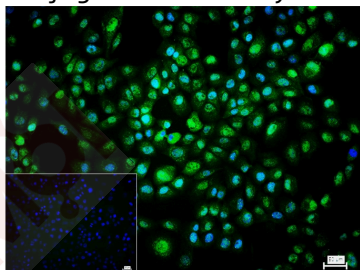
6. Paraformaldehyde-fixed, paraffin embedded Human Colon Cancer; Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Antibody incubation with Smad3 Monoclonal Antibody, Unconjugated (TMAB-01740) at 1:200 overnight at 4°C, followed by conjugation to the secondary antibody and DAB staining.

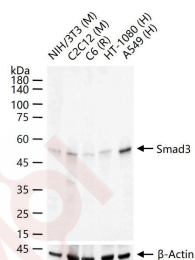
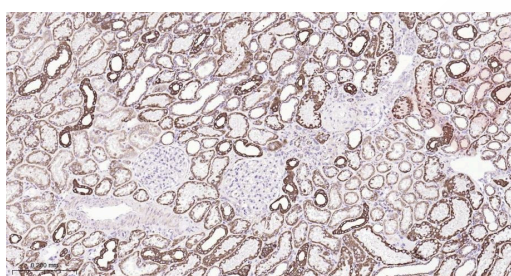
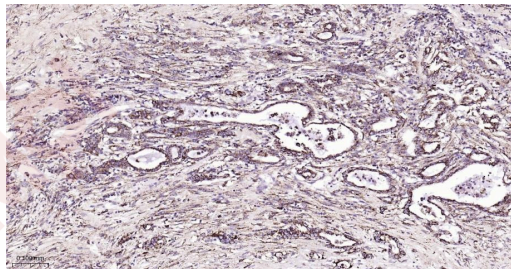
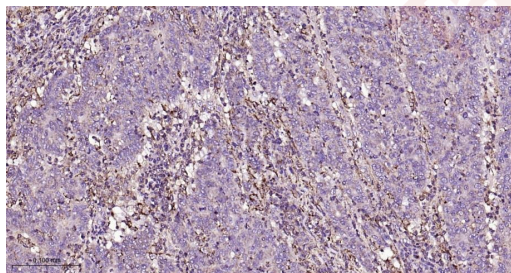
7. Paraformaldehyde-fixed, paraffin embedded Human Pancreatic Cancer; Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Antibody incubation with Smad3 Monoclonal Antibody, Unconjugated (TMAB-01740) at 1:200 overnight at 4°C, followed by conjugation to the secondary antibody and DAB staining.

Verified Activity:

8. Paraformaldehyde-fixed, paraffin embedded Human Kidney; Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Antibody incubation with Smad3 Monoclonal Antibody, Unconjugated (TMAB-01740) at 1:200 overnight at 4°C, followed by conjugation to the secondary antibody and DAB staining.

9. 25 µg total protein per Lane of various lysates probed with Smad3 monoclonal antibody, unconjugated (TMAB-01740) at 1:1000 dilution and 4°C overnight incubation. Followed by conjugated secondary antibody incubation at RT for 60 min.





Application: FCM,ICC/IF,IF,IHC-Fr,IHC-P,WB

Recommended WB: 1:500-2000; IHC-P: 1:100-500; IHC-Fr: 1:100-500; ICC/IF: 1:50-200; IF: 1:100-500; FCM: 1ug/Test

### Properties

Stability & Storage: Store at -20°C or -80°C for 12 months. Avoid repeated freeze-thaw cycles.

Shipping: Shipping with blue ice.

### Antigen Details

Immunogen: A synthesized peptide: human SMAD3

Antigen Species: Human

Gene ID: 4088

Uniprot ID: P84022

Synonyms: JV15-2;SMAD3;Mothers against decapentaplegic homolog 3;hMAD-3;SMAD family member 3 (SMAD 3;Smad3;hSMAD3);Mothers against DPP homolog 3;Mad3;MADH3;MAD homolog 3

Biology Area: Response to hypoxia,SMAD family,SMADs,Hypoxia,Cancer,SMADs,Cytoplasmic

### Research Background

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.

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