

## Anti-SMAD3 Polyclonal Antibody

### Product Details

Ig Type:	IgG
Reactivity:	Human,Mouse,Rat
Molecular Weight:	Theoretical: 47 kDa. Actual: 54 kDa.
Purification:	Protein A purified

### Applications

1. Blank control (Black line): HUVEC (Black).  
Primary Antibody (green line): Rabbit Anti-Smad3 antibody (TMAB-01739)  
Dilution: 1 µg/10<sup>6</sup> cells;  
Isotype Control Antibody (orange line): Rabbit IgG.  
Secondary Antibody (white blue line): Goat anti-rabbit IgG-AF647  
Dilution: 1 µg/test.

#### Protocol

The cells were fixed with 4% PFA (10 min 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.

#### 2. Sample:

Cerebrum (Mouse) Lysate at 40 µg  
Ovary (Mouse) Lysate at 40 µg  
HT1080 (Human) Cell Lysate at 30 µg  
Jurkat (Human) Cell Lysate at 30 µg  
Primary: Anti-Smad3 (TMAB-01739) at 1/1000 dilution  
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution

#### Verified Activity:

Predicted band size: 52 kDa  
Observed band size: 60 kDa

#### 3. Blank control: Hela.

Primary Antibody (green line): Rabbit Anti-Smad3 antibody (TMAB-01739)  
Dilution: 1 µg/10<sup>6</sup> cells;  
Isotype Control Antibody (orange line): Rabbit IgG.  
Secondary Antibody: Goat anti-rabbit IgG-AF647  
Dilution: 1 µg/test.

#### Protocol

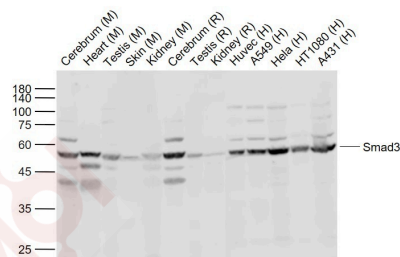
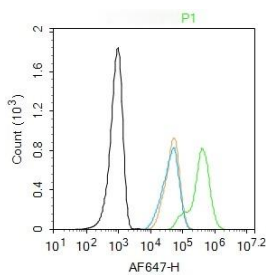
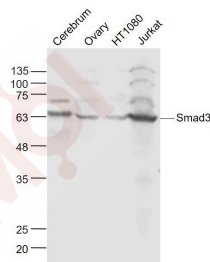
The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with 90% ice-cold methanol for 20 min at -20°C. The cells were then incubated in 5% BSA to block non-specific protein-protein interactions for 30 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature.

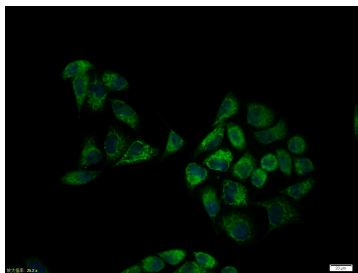
#### 4. Sample:

Lane 1: Cerebrum (Mouse) Lysate at 40 µg  
Lane 2: Heart (Mouse) Lysate at 40 µg  
Lane 3: Testis (Mouse) Lysate at 40 µg  
Lane 4: Skin (Mouse) Lysate at 40 µg

Lane 5: Kidney (Mouse) Lysate at 40 µg  
 Lane 6: Cerebrum (Rat) Lysate at 40 µg  
 Lane 7: Testis (Rat) Lysate at 40 µg  
 Lane 8: Kidney (Rat) Lysate at 40 µg  
 Lane 9: Huvec (Human) Cell Lysate at 30 µg  
 Lane 10: A549 (Human) Cell Lysate at 30 µg  
 Lane 11: Hela (Human) Cell Lysate at 30 µg  
 Lane 12: HT1080 (Human) Cell Lysate at 30 µg  
 Lane 13: A431 (Human) Cell Lysate at 30 µg  
 Primary: Anti-Smad3 (TMAB-01739) at 1/1000 dilution  
 Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution  
 Predicted band size: 52 kDa  
 Observed band size: 54 kDa

5. Hela 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) polyclonal Antibody, Unconjugated (TMAB-01739) 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.





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

Recommended FCM=1 µg/Test; ICC/IF=1:100-500; IF=1:100-500; IHC-Fr=1:100-500; IHC-P=1:100-500; WB=1:500-2000

### 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: KLH conjugated synthetic peptide: human Smad3

Antigen Species: Human

Gene ID: 4088

Uniprot ID: P84022

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

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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