

## Anti-Phospho-Smad3 (Ser208) Polyclonal Antibody

### Product Details

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

### Applications

1. Paraformaldehyde-fixed, paraffin embedded (Rat brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Phospho-Smad3 (Ser208)) Polyclonal Antibody, Unconjugated (TMAB-11299) at 1:500 overnight at 4°C, followed by a conjugated secondary for 20 minutes and DAB staining.

2. Sample:

Lane 1: Huvec (Human) Cell Lysate at 30 µg

Lane 2: Hela (Human) Cell Lysate at 30 µg

Lane 3: HT1080 (Human) Cell Lysate at 30 µg

Lane 4: A431 (Human) Cell Lysate at 30 µg

Lane 5: A549 (Human) Cell Lysate at 30 µg

Primary: Anti-Phospho-Smad3 (Ser208) (TMAB-11299) at 1/1000 dilution

Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution

Verified Activity: Predicted band size: 52 kD

Observed band size: 54 kD

3. Blank control: A431.

Primary Antibody (green line): Rabbit Anti-Phospho-Smad3 (Ser208) antibody (TMAB-11299)

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. Acquisition of 20,000 events was performed.

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

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

## A DRUG SCREENING EXPERT

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### Properties

Stability & Storage: Store at 2°C-8°C for 1 month. Store at -20°C or -80°C for 12 months. Avoid repeated freeze-thaw cycles.

Shipping: Shipping with blue ice.

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### Antigen Details

Immunogen: KLH conjugated Synthesised phosphopeptide: human Smad3 around the phosphorylation site of Ser208

Antigen Species: Human

Gene ID: 4088

Uniprot ID: P84022

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### 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.

**Inhibitor · Natural Compounds · Compound Libraries · Recombinant Proteins**

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