Data Sheet (Cat.No.TMAB-11296)

Anti-Phospho-SMAD2 (Ser464) Polyclonal Antibody

Product Details

Ig Type:	IgG
Reactivity:	Human,Mouse (predicted:Rat,Chicken,Dog,Cow,Horse)
Molecular Weight:	Theoretical: 58 kDa. Actual: 58 kDa.
Purification:	Protein A purified

Applications

Verified Activity:	1. Sample: Lane 1: Hela (Human) Cell Lysate at 30 µg Lane 2: HT1080 (Human) Cell Lysate at 30 µg Lane 3: Jurkat (Human) Cell Lysate at 30 µg Lane 4: A549 (Human) Cell Lysate at 30 µg Lane 5: 293T (Human) Cell Lysate at 30 µg Primary: Anti-phospho-SMAD2 (Ser464) (TMAB-11296) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 60 kD Observed band size: 60 kD 2. Paraformaldehyde-fixed, paraffin embedded (mouse 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-SMAD2 (Ser464)) Polyclonal Antibody, Unconjugated (TMAB-11296) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining. 3. Paraformaldehyde-fixed, paraffin embedded (human placenta); 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-SMAD2 (Ser464)) Polyclonal Antibody, Unconjugated (TMAB-11296) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining. 4. Blank control (Black line): Mouse spleen (Black). Primary Antibody (green line): Rabbit Anti-phospho-SMAD2 (Ser425) antibody Dilution: 3 µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody (white blue line): Goat anti-rabbit IgG-PE 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 goat serum to block non-specific protein-protein interactions for 15 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. 5. Sample: Lane1: Heart (Mouse) Lysate at 40 µg Lane2: Muscle (Mouse) Lysate at 40 µg

	Primary: Anti-phospho-SMAD2 (Ser464) (TMAB-11296) at 1/300 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 58 kD Observed band size: 58 kD
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: 3μg/Test

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.

Antigen Details

Immunogen:	KLH conjugated synthesised phosphopeptide: human Smad2 around the phosphorylation site of Ser464
Antigen Species:	Human
Gene ID:	4087
Uniprot ID:	Q15796

Research Background

The protein encoded by this gene belongs to the SMAD, a family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (Mad) and the C. elegans gene Sma. SMAD proteins are signal transducers and transcriptional modulators that mediate multiple signaling pathways. This protein mediates the signal of the transforming growth factor (TGF)-beta, and thus regulates multiple cellular processes, such as cell proliferation, apoptosis, and differentiation. This protein is recruited to the TGF-beta receptors through its interaction with the SMAD anchor for receptor activation (SARA) protein. In response to TGF-beta signal, this protein is phosphorylated by the TGF-beta receptors. The phosphorylation induces the dissociation of this protein with SARA and the association with the family member SMAD4. The association with SMAD4 is important for the translocation of this protein into the nucleus, where it binds to target promoters and forms a transcription repressor complex with other cofactors. This protein can also be phosphorylated by activin type 1 receptor kinase, and mediates the signal from the activin. Alternatively spliced transcript variants have been observed for this gene. [provided by RefSeq, May 2012]