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[Primary Antibody]

Rabbit Anti-phospho-Smad2 (Ser465)antibody

Catalog Number: SL2224R

FOR RESEARCH USE ONLY

[DATASHEET]

Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Target:	phospho-Smad2 (Ser465)
Immunogen:	KLH conjugated Synthesised phosphopeptide derived from human Smad2 around the phosphorylation site of Ser465: CS(p-S)MS
Purification:	affinity purified by Protein A
Concentration:	1mg/ml
Storage:	0.01M TBS (pH7.4) with 1% BSA, 0.02% Proclin300 and 50% Glycerol. Shipped at 4°C. Store at -20 °C for one year. Avoid repeated freeze/thaw cycles.
Applications:	WB=1:500-5000 Flow-Cyt=1μg/Test （Paraffin sections need antigen repair） not yet tested in other applications. optimal dilutions/concentrations should be determined by the end user.
Reactivity:	Human,Mouse(predicted:Rat,Chicken,Dog,Cow,Horse)
Predicted MW.:	58kDa
Subcellular Location:	The nucleus cytoplasmic
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

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	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] **Function:** Receptor-regulated SMAD (R-SMAD) that is an intracellular signal transducer and transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinases. Binds the TRE element in the promoter region of many genes that are regulated by TGF-beta and, on formation of the SMAD2/SMAD4 complex, activates transcription. May act as a tumor suppressor in colorectal carcinoma. Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator. **Subunit:** Momomer; the absence of TGF-beta. Heterodimer; in the presence of TGF-beta. Forms a heterodimer with co-SMAD, SMAD4, in the nucleus to form the transactivation complex SMAD2/SMAD4. Interacts with AIP1, HGS, PML and WWP1. Interacts with NEDD4L in response to TGF-beta. Found in a complex with SMAD3 and TRIM33 upon addition of TGF-beta. Interacts with ACVR1B, SMAD3 and TRIM33. Interacts (via the MH2 domain) with ZFYVE9; may form trimers with the SMAD4 co-SMAD. Interacts with FOXH1, homeobox protein TGIF, PEBP2-alpha subunit, CREB-binding protein (CBP), EP300 and SKI. Interacts with SNON; when phosphorylated at Ser-465/467. Interacts with SKOR1 and SKOR2. Interacts with PRDM16. Interacts (via MH2 domain) with LEMD3. Interacts with RBPMS. Interacts with WWP1. Interacts (dephosphorylated form, via the MH1 and MH2 domains) with RANBP3 (via its C-terminal R domain); the interaction results in the export of dephosphorylated SMAD3 out of the nucleus and termination ot the TGF-beta signaling. Interacts with PDPK1 (via PH domain). **Subcellular Location:** Cytoplasm. Nucleus. Note=Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4. On dephosphorylation by phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1. **Tissue Specificity:** Expressed at high levels in skeletal muscle, heart and placenta. **Post-translational modifications:** Phosphorylated on one or several of Thr-220, Ser-245, Ser-250, and Ser-255. In response to TGF-beta, phosphorylated

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	on Ser-465/467 by TGF-beta and activin type 1 receptor kinases. Able to interact with SMURF2 when phosphorylated on Ser-465/467, recruiting other proteins, such as SNON, for degradation. In response to decorin, the naturally occurring inhibitor of TGF-beta signaling, phosphorylated on Ser-240 by CaMK2. Phosphorylated by MAPK3 upon EGF stimulation; which increases transcriptional activity and stability, and is blocked by calmodulin. Phosphorylated by PDPK1. In response to TGF-beta, ubiquitinated by NEDD4L; which promotes its degradation. Acetylated on Lys-19 by coactivators in response to TGF-beta signaling, which increases transcriptional activity. Isoform short: Acetylation increases DNA binding activity in vitro and enhances its association with target promoters in vivo. Acetylation in the nucleus by EP300 is enhanced by TGF-beta. **Similarity:** Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain. **Database links:** **Entrez Gene:** 4087 Human **Entrez Gene:** 17126 Mouse **Entrez Gene:** 29357 Rat Omim: 601366 Human **SwissProt:** Q15796 Human **SwissProt:** Q62432 Mouse **SwissProt:** O70436 Rat **Unigene:** 12253 Human **Unigene:** 705764 Human **Unigene:** 391091 Mouse **Unigene:** 2755 Rat transcriptional regulatory factor（Transcriptin Regulators） The Smads family is a recently discovered important new gene family in the TGF-β Signal transduction pathway. It is a TGF-β-specific intracellular Signal transduction molecule, and SMAD2/3 belong to the receptor-activated SMADs. Some scholars believe that mutations in Smad2 and Smad3 in certain Tumour types may indicate that they are Tumour suppressor genes. Smad2/3 proteins have high homology with Smad1 and Smad5.

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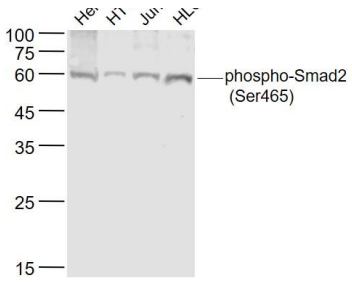
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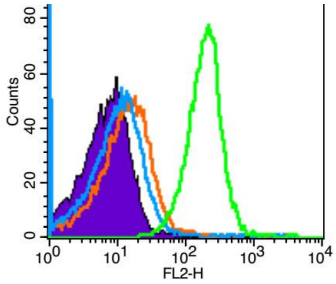
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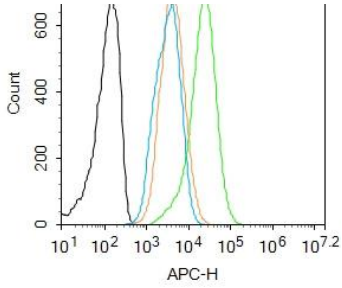
[VALIDATION IMAGES]



Sample:
Lane 1: Hela (Human) Cell Lysate at 30 ug
Lane 2: HT1080 (Human) Cell Lysate at 30 ug
Lane 3: Jurkat (Human) Cell Lysate at 30 ug
Lane 4: HL60 (Human) Cell Lysate at 30 ug
Primary: Anti-phospho-Smad2 (Ser465) (SL2224R) at 1/1000 dilution
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution
Predicted band size: 60 kD
Observed band size: 60 kD



Blank control (Black line): Raji (Black). Primary Antibody (green line): Rabbit Anti-phospho-Smad2 (Ser465) antibody (SL2224R) Dilution: 3µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG .
Secondary Antibody (white blue line): Goat anti-rabbit IgG-PE Dilution: 1µg /test.
Protocol The cells were fixed with 4% PFA (10min 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.



Blank control: Hela. Primary Antibody (green line): Rabbit Anti-phospho-Smad2 (Ser465) antibody (SL2224R) Dilution: 1µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG .
Secondary Antibody : Goat anti-rabbit IgG-AF647 Dilution: 1µg /test.
Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 90% ice-cold methanol for 20 min at -20℃. 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.

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