10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Phospho-SMAD3(S208) Antibody

Affinity Purified Rabbit Polyclonal Antibody (Pab)
Catalog # AP3249a

Product Information

Application	IF, DB, E
Primary Accession	P84022
Other Accession	P84025, P84024, Q8BUN5, P84023
Reactivity	Human
Predicted	Chicken, Mouse, Pig, Rat
Host	Rabbit
Clonality	Polyclonal
Isotype	Rabbit IgG
Calculated MW	48081

Additional Information

Gene ID	4088
Other Names	Mothers against decapentaplegic homolog 3, MAD homolog 3, Mad3, Mothers against DPP homolog 3, hMAD-3, JV15-2, SMAD family member 3, SMAD 3, Smad3, hSMAD3, SMAD3, MADH3
Target/Specificity	This SMAD3 Antibody is generated from rabbits immunized with a KLH conjugated synthetic phosphopeptide corresponding to amino acid residues surrounding S208 of human SMAD3.
Dilution	IF~~1:10~50 DB~~1:500 E~~Use at an assay dependent concentration.
Format	Purified polyclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is purified through a protein A column, followed by peptide affinity purification.
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Precautions	Phospho-SMAD3(S208) Antibody is for research use only and not for use in diagnostic or therapeutic procedures.

Protein Information

Name	SMAD3
Synonyms	MADH3
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 SMAD3/SMAD4 complex, activates transcription. Also can form a SMAD3/SMAD4/JUN/FOS complex at the AP- 1/SMAD site to regulate TGF-beta-mediated transcription. Has an inhibitory effect on wound healing probably by modulating both growth and migration of primary keratinocytes and by altering the TGF-mediated chemotaxis of monocytes. This effect on wound healing appears to be hormone-sensitive. Regulator of chondrogenesis and osteogenesis and inhibits early healing of bone fractures. Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator.
Cellular 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 (PubMed:15799969, PubMed:21145499). Through the action of the phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1 (PubMed:16751101, PubMed:19289081). Co-localizes with LEMD3 at the nucleus inner membrane (PubMed:15601644). MAPK-mediated phosphorylation appears to have no effect on nuclear import (PubMed:19218245). PDPK1 prevents its nuclear translocation in response to TGF-beta (PubMed:17327236). Localized mainly to the nucleus in the early stages of embryo development with expression becoming evident in the cytoplasm of the inner cell mass at the blastocyst stage (By similarity) {ECO:0000250|UniProtKB:Q8BUN5, ECO:0000269|PubMed:15601644, ECO:0000269|PubMed:15799969, ECO:0000269|PubMed:16751101, ECO:0000269|PubMed:17327236, ECO:0000269|PubMed:19218245, ECO:0000269|PubMed:19289081, ECO:0000269|PubMed:21145499}

Background

SMAD3, a receptor regulated SMAD (R-SMAD) is a transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinase. SMAD3 is estimated to account for at least 80% of all TGF-beta-mediated response. Activated type I receptor phosphorylates receptor-activated SMADS (RSMADS) at their c-terminal two extreme serines in the SSXS motif. The phosphorylated R-SMAD translocate into nucleus, where they regulate transcription of target genes. SMAD3 signal transduction appears to be important in the rgulation of muscle-specific genes. Loss of SMAD3 is a feature of pediatric T-cell lymphoblastic leukemia, while upregulation of SMAD3 may be responsible for TGFB hyperresponsiveness observed in scleroderma.

References

References for protein:
1.Imoto, S., et al., FEBS Lett. 579(13):2853-2862 (2005).
2.Dubrovska, A., et al., Oncogene 24(14):2289-2297 (2005).
3.Furumatsu, T., et al., J. Biol. Chem. 280(9):8343-8350 (2005).
4.Kobayashi, T., et al., Biochem. Biophys. Res. Commun. 327(2):393-398 (2005).
5.Kamaraju, A.K., et al., J. Biol. Chem. 280(2):1024-1036 (2005).

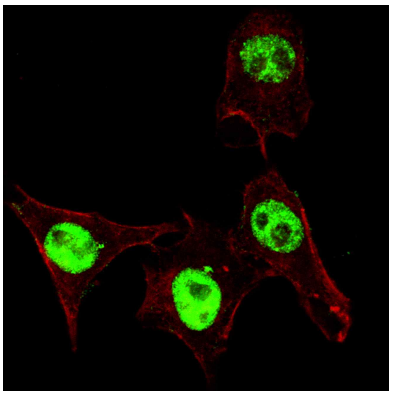
References for HeLa cell line:
1. Scherer WF, Syverton JT, Gey GO (May 1953). "Studies on the propagation in vitro of poliomyelitis viruses. IV. Viral multiplication in a stable strain of human malignant epithelial cells (strain HeLa) derived from an epidermoid carcinoma of the cervix". J. Exp. Med. 97 (5): 695–710. [PubMed:13052828].

2. Macville M, Schr□ck E, Padilla-Nash H, Keck C, Ghadimi BM, Zimonjic D, Popescu N, Ried T (January 1999). "Comprehensive and definitive molecular cytogenetic characterization of HeLa cells by spectral karyotyping".

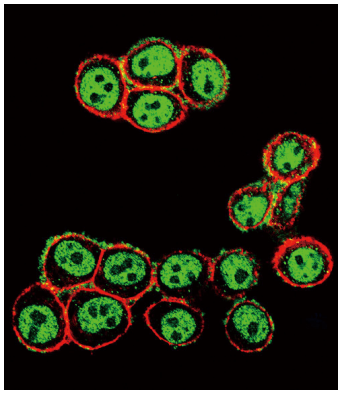
Cancer Res. 59 (1): 141–50. [PubMed: 9892199].
3. Rahbari R, Sheahan T, Modes V, Collier P, Macfarlane C, Badge RM (April 2009). "A novel L1 retrotransposon marker for HeLa cell line identification". BioTechniques 46 (4): 277–84. [PubMed: 19450234].

4. Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, MacLeod RA, Masters JR, Nakamura Y, Reid YA, Reddel RR, Freshney RI (July 2010). "Check your cultures! A list of cross-contaminated or misidentified cell lines". Int. J. Cancer 127 (1): 1–8. [PubMed:20143388].

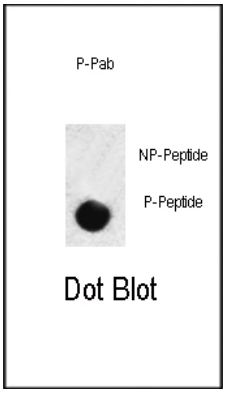
Images



Fluorescent confocal image of HeLa cells stained with phospho-SMAD3-S208 antibody. HeLa cells were fixed with 4% PFA (20 min), permeabilized with Triton X-100 (0.2%, 30 min). Cells were then incubated with AP3249a phospho-SMAD3-S208 primary antibody (1:200, 2 h at room temperature). For secondary antibody, Alexa Fluor® 488 conjugated donkey anti-rabbit antibody (green) was used (1:1000, 1h). Cytoplasmic actin was counterstained with Alexa Fluor® 555 (red) conjugated Phalloidin (5.25 µM, 25 min). Pictures were taken on a Biorevo microscope (BZ-900, Keyence).Note the highly specific localization of the phospho-SMAD3 mainly to the nucleus, supported by Human Protein Atlas Data (http://www.proteinatlas.org/ENSG00000166949).



Confocal immunofluorescent analysis of Phospho-SMAD3-S208 Antibody(Cat#AP3249a) with Hela cell followed by Alexa Fluor 488-conjugated goat anti-rabbit lgG (green). Actin filaments have been labeled with Alexa Fluor 555 phalloidin (red).



Dot blot analysis of anti-hSMAD3-S208 Phospho-specific Pab (Cat. #AP3249a) on nitrocellulose membrane. 50ng of Phospho-peptide or Non Phospho-peptide per dot were adsorbed. Antibody working concentrations are 0.5ug per ml.

Citations

- Constitutive Smad linker phosphorylation in melanoma: a mechanism of resistance to transforming growth factor-β-mediated growth inhibition.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.