

**Phospho-SMAD3(S208) Antibody**  
**Affinity Purified Rabbit Polyclonal Antibody (Pab)**  
**Catalog # AP3249a****Specification**

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**Phospho-SMAD3(S208) Antibody - Product Information**

Application	DB, IF, E
Primary Accession	<a href="#">P84022</a>
Other Accession	<a href="#">P84025</a> , <a href="#">P84024</a> , <a href="#">Q8BUN5</a> , <a href="#">P84023</a>
Reactivity	Human
Predicted	Chicken, Mouse, Pig, Rat
Host	Rabbit
Clonality	Polyclonal
Isotype	Rabbit IgG

**Phospho-SMAD3(S208) Antibody - 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**

DB~~1:500

IF~~1:10~50

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.

**Phospho-SMAD3(S208) Antibody - Protein Information****Name** SMAD3 ([HGNC:6769](#))

## 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

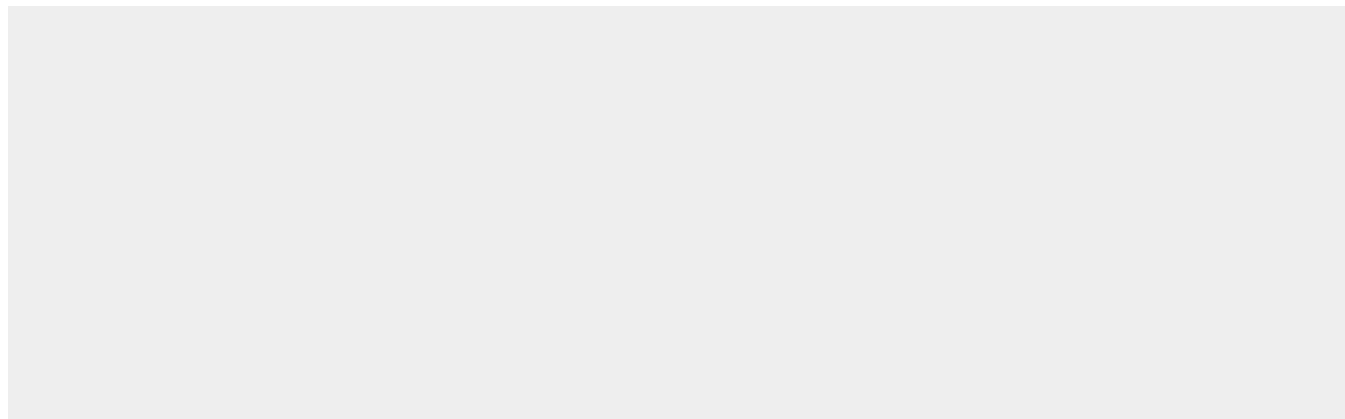
Cytoplasm. Nucleus. Note=Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4 (PubMed:15799969, PubMed:21145499). Through the action of the phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus 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}

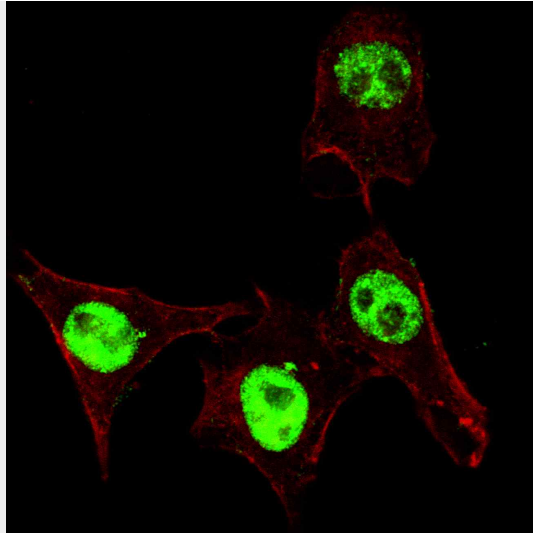
## Phospho-SMAD3(S208) Antibody - Protocols

Provided below are standard protocols that you may find useful for product applications.

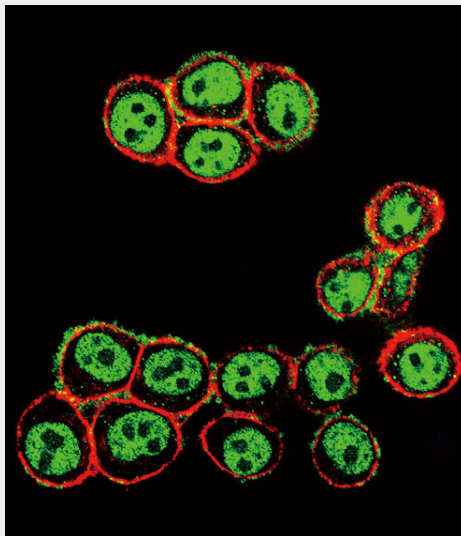
- [Western Blot](#)
- [Blocking Peptides](#)
- [Dot Blot](#)
- [Immunohistochemistry](#)
- [Immunofluorescence](#)
- [Immunoprecipitation](#)
- [Flow Cytometry](#)
- [Cell Culture](#)

## Phospho-SMAD3(S208) Antibody - Images

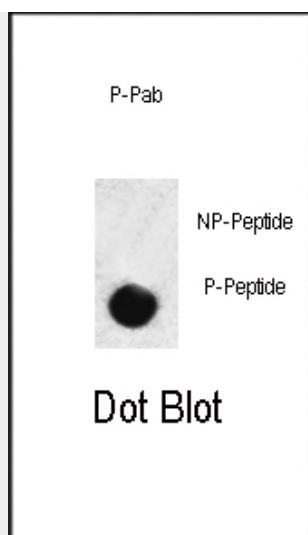




Fluorescent confocal image of HeLa cells stained with phospho-SMAD3-S208 antibody. HeLa cells were fixed with 4% PFA (20 min), permeabilized with Triton X-100 (0.2%, 30 min). Cells were then incubated with AP3249a phospho-SMAD3-S208 primary antibody (1:200, 2 h at room temperature). For secondary antibody, 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  $\mu$ M, 25 min). Pictures were taken on a Bioevo 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 cells followed by Alexa Fluor 488-conjugated goat anti-rabbit IgG (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.

### **Phospho-SMAD3(S208) Antibody - 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.

### **Phospho-SMAD3(S208) Antibody - References**

References for protein:

- 1.Imoto, S., et al., FEBS Lett. 579(13):2853-2862 (2005).
- 2.Dubrovskaja, 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, Schröck 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].

**Phospho-SMAD3(S208) Antibody - Citations**

- [Constitutive Smad linker phosphorylation in melanoma: a mechanism of resistance to transforming growth factor- \$\beta\$ -mediated growth inhibition.](#)