Datasheet for 600-401-C48

SMAD3 phospho T179 Antibody

Overview

Description:	Anti-SMAD3 pT179 (RABBIT) Antibody - 600-401-C48
Item No.:	600-401-C48
Size:	100 µg
Applications:	ELISA, WB, IHC, IP
Reactivity:	Mouse
Host Species:	Rabbit

Product Details

Background:	SMAD3 pT179 is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI) and is suitable for Cancer, Immunology and Nuclear Signaling research. Smad3 (also known as Mothers against decapentaplegic homolog 3, Mothers against DPP homolog 3, Mad3, hMAD-3, JV15-2 or hSMAD3) is a transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinase. These activators exert diverse effects on a wide array of cellular processes. The Smad proteins mediate much of the signaling responses induced by the TGF-beta superfamily. Activated type I receptor phosphorylates receptor-activated Smads (R-Smads) at their c-terminal two extreme serines in the S-S-X-S motif, e.g. Smad2 and Smad3 proteins in the TGF-b pathway, or Smad1, Smad5 or Smad8 in the bone morphogenic protein or BMP pathway. The phosphorylated R-Smads are translocated into nucleus, where they regulate transcription of target genes. Based on microarray and animal model experiments, Smad3 accounts for at least 80% of all TGF-b-mediated response.
Synonyms:	rabbit anti-SMAD3 pT179 antibody, SMAD-3, SMAD 3, mothers against decapentaplegic homolog 3 antibody, MAD homolog 3, Mothers against DPP homolog 3, SMAD family member 3, MADH3, MADH 3, JV15-2
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	SMAD3

Reactivity:	Mouse
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-SMAD3 pT179 antibody was prepared by repeated immunizations with a synthetic phospho-peptide corresponding to an internal region of human Smad3 protein surrounding amino acid residue 179.
Purity/Specificity:	Anti-SMAD3 pT179 affinity-purified antibody is directed against the phosphorylated form of human Smad3 protein at the pT179 residue. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross adsorbed against the non-phosphorylated form of the immunizing peptide. Reactivity occurs against human Smad3 pT179 protein and the antibody is specific for the phosphorylated form of the protein. Reactivity with non-phosphorylated human Smad3 is minimal by ELISA and western blot. Expect reactivity against phosphorylated Smad2. Reactivity against other phosphorylated Smad family members is not known. A BLAST analysis was used to suggest cross reactivity with Smad3 from human, mouse, rat, pig, dog, and marmoset based on 100% sequence homology with the immunogen. Reactivity against homologues from other sources is not known.
Relevant Links:	• UniProtKB - P84022 • NCBI - NP_005893 • GeneID - 4088

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IHC, IP (Based on references)
Application Note:	Anti-SMAD3 pT179 has been tested for use in ELISA and by western blot, and suitable by immunohistochemistry. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 48.1 kDa in size corresponding to human phosphorylated Smad3 protein by western blotting in the appropriate stimulated tissue or cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:15,000-1:75,000
WB:	1:1,000

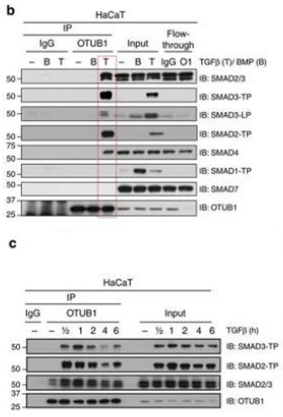
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

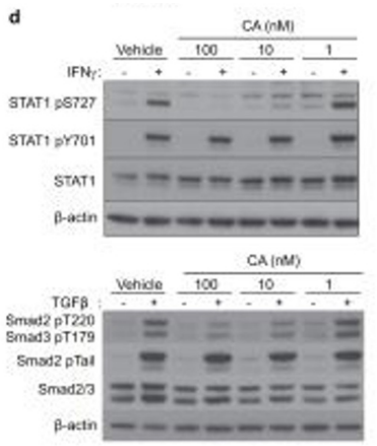
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

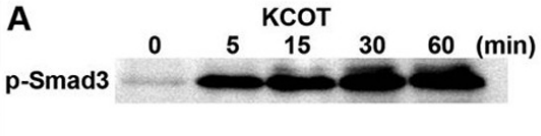
Images



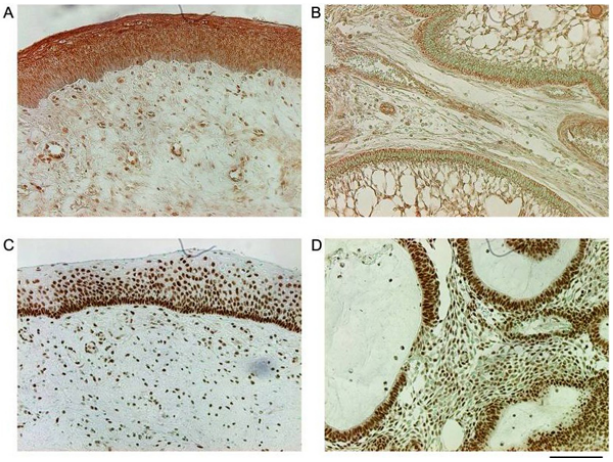
Western Blot
(b) An endogenous IP with OTUB1 antibody or pre-immune sheep IgG was performed in HaCaT cell extracts, stimulated without or with 50 pM TGFβ or 25 ng ml−1 BMP for 1 h. Cell extracts (input), endogenous IgG or OTUB1 IPs and the corresponding immune-depleted flow-through extracts (O1=OTUB1-depleted untreated HaCaT extract) were resolved by SDS-PAGE and immunoblotted (IB) with the indicated antibodies [Anti-phospho-Ser423/425 SMAD3 (SMAD3-TP; p/n 600-401-919) and anti-phospho-Thr179 SMAD3 (SMAD3-LP; p/n 600-401-C48)]. (c) As in b, except that a time course of 50 pM TGFβ treatment was performed for up to 6 h before lysis. Fig 1. PMID: 24071738



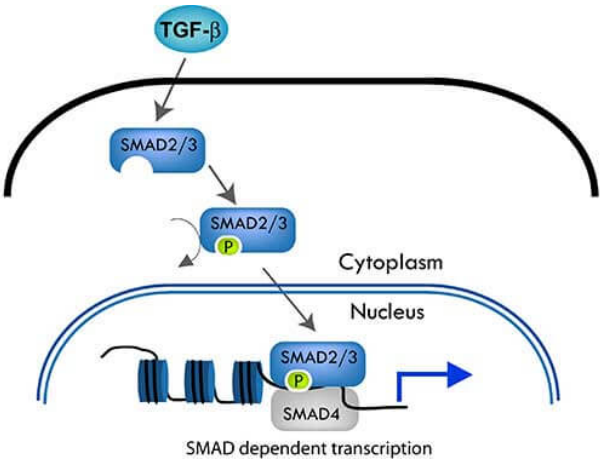
Western Blot
(d) Immunoblots showing CA inhibition of CDK8-dependent IFN-γ-stimulated STAT1 S727 phosphorylation in MOLM-14 cells and CA inhibition of TGF-β-stimulated Smad2 T220 and Smad3 T179 phosphorylation (p/n 600-401-C48) in HaCaT cells (IC50 < 100 nM). Extended Data Figure 2.
Full scan in Supplementary Figure 1. PMID: 26416749



Western Blot
Effects of phosphorylation of Smad3 in KCOT stromal fibroblasts, and effects of anti-TGF-β antibody and TGF-β receptor inhibitor on KCOT fluid-induced RANKL expression in KCOT stromal fibroblasts. (A) KCOT stromal fibroblasts were stimulated with 1% KCOT fluid, and phosphorylation of Smad3 (p/n 600-401-C48) was examined by western blotting. KCOT fluid stimulation was found to induce phosphorylation of Smad3. Fig 3. PMID: 27279422

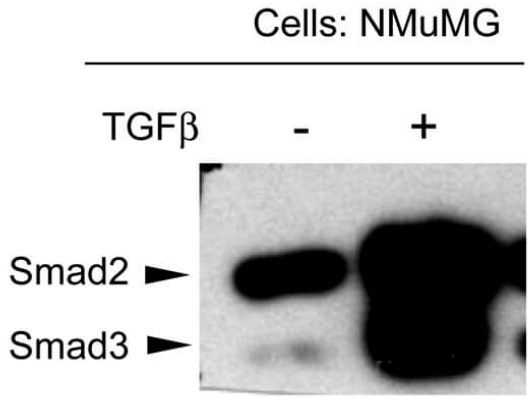


Immunohistochemistry
Immunohistochemical staining for TGF-β and phosphorylated Smad3 (p/n 600-401-C48). Representative staining results for TGF-β (A and B) and phosphorylated Smad3 (C and D) in the KCOT (A and C) and ameloblastoma (B and D) are shown. The bar represents 50 µm. Fig 6.
PMID: 27279422



Pathway

The SMAD pathway follows the canonical TGF-ß signaling pathway. TGF-ß dimers bind to a receptor thereby activating the pathway. The type I receptor then recruits and phosphorylates a receptor regulated SMAD (R-SMAD).i.e. SMAD2 or SMAD3. The R-SMAD then binds to the common SMAD (coSMAD) i.e. SMAD4, and forms a heterodimeric complex. This complex then enters the cell nucleus and acts as a transcription factor.



Western Blot

NMuMG mouse mammary epithelial cells were probed for the activation of Smad3 by detecting phosphorylation of threonine 179. The cells were either untreated or treated with TGF-beta, transferred to membranes and probed with Anti-SMAD3 pT179 (RABBIT) Antibody. The antibody detects only Smad3 in stimulated cells suggesting detection of phosphorylated SMAD3 at T179.

References

- Tripathi V, Sixt KM, Gao S, et al Direct Regulation of Alternative Splicing by SMAD3 through PCBP1 Is Essential to the Tumor-Promoting Role of TGF-β. *Mol Cell.* (2016)
- Feng et al. Discovery of a Small-Molecule BMP Sensitizer for Human Embryonic Stem Cell Differentiation. *Cell Reports* (2016)
- Yamada, C et al. TGF-β in jaw tumor fluids induces RANKL expression in stromal fibroblasts. *International Journal of Oncology* (2016)
- Pelish et al. Mediator kinase inhibition further activates super-enhancer-associated genes in AML. *Nature* (2015)
- Herhaus L. et al. OTUB1 enhances TGFβ signalling by inhibiting the ubiquitylation and degradation of active SMAD2/3. *Nat Commun.* (2013)

Disclaimer

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