





SMAD3 Polyclonal Antibody

Catalog Number 51-1500 Product data sheet

Details	
Size	100 μg
Host/Isotope	Rabbit / IgG
Class	Polyclonal
Туре	Antibody
Immunogen	A 20 amino acid synthetic peptide derived from a central portion of the linker domain of human Smad3
Conjugate	Unconjugated
Form	Liquid
Concentration	0.25 mg/mL
Purification	Antigen affinity chromatography
Storage buffer	PBS, pH 7.4
Contains	0.1% sodium azide
Storage Conditions	-20°C

Species Reactivity	
Species reactivity	Human
Published species	Dog, Tag, Mink, Rat, Non-human primate, Virus, Deer, Luciferase, Human, Mouse, Not Applicable

Tested Applications	Dilution *
ChIP assay (ChIP)	2 μg/10^6 cells
ELISA (ELISA)	0.1-1.0 μg/mL
Immunohistochemistry (IHC)	1.0 µg/mL
Western Blot (WB)	1-3 µg/mL
Immunocytochemistry (ICC/IF)	1:100-1:500
Published Applications	

Published Applications		
Immunocytochemistry (ICC/IF)	See 8 publications below	
Western Blot (WB)	See 25 publications below	
Immunohistochemistry (IHC)	See 6 publications below	
Immunohistochemistry (Paraffin) (IHC (P))	See 2 publications below	
Miscellaneous PubMed (Misc)	See 3 publications below	
ChIP assay (ChIP)	See 1 publications below	
Gel Shift (GS)	See 1 publications below	

^{*} Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Background/Target Information

SMAD3 belongs to the SMAD family of proteins. They are 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 functions as a transcriptional modulator activated by transforming growth factor-beta and is thought to play a role in the regulation of carcinogenesis.

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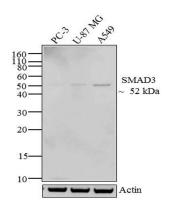
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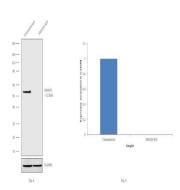


Product Images For SMAD3 Polyclonal Antibody



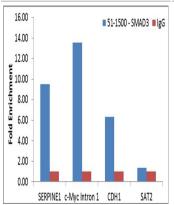
SMAD3 Antibody (51-1500) in WB

Western blot analysis of SMAD3 was performed by loading 35 µg of PC-3 (lane1), U-87 MG (lane2) and a549 (lane3) cell lysate using Novex®NuPAGE® 12 % Bis-Tris gel (Product # NP0341BOX), XCell SureLock Electrophoresis System (Product # El0002), Novex® Sharp Pre-Stained Protein Standard (LC5800), and iBlot® Dry Blotting System (IB21001). Proteins were transferred to a nitrocellulose membrane and blocked with 5% skim milk for 1 hour at room temperature. SMAD3 was detected at ~ 52 kDa using SMAD3 Rabbit Polyclonal Antibody (Product # 51-1500) at 1-3 µg/mL in 5% skim milk at 4°C overnight on a rocking platform. Goat Anti-Rabbit IgG - HRP Secondary Antibody (G21234) at 1:5000 dilution was used and chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



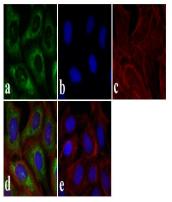
SMAD3 Antibody (51-1500)

Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. Loss of signal was observed for target protein in SMAD3 knockout (KO) cell line using Anti-SMAD3 polyclonal Antibody (Product # 51-1500). {KO}



SMAD3 Antibody (51-1500)

Antibody specificity was demonstrated by detection of enrichment of the target protein at specific gene loci. Chromatin Immunoprecipitation (ChIP) was performed using Anti-SMAD3 Rabbit Polyclonal Antibody (Product # 51-1500) using PCR primer pairs over SERPINE1, c-Myc Intron 1 and CDH1 (active) and SAT2 satellite repeats (inactive). {RE}



SMAD3 Antibody (51-1500) in ICC/IF

Immunofluorescence analysis of SMAD3 was done on 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.25% Triton™ X-100 for 10 minutes, and blocked with 5% BSA for 1 hour at room temperature. The cells were labeled with SMAD3 Rabbit polyclonal Antibody (Product # 51-1500) at 2 μg/mL in 1% BSA and incubated for 3 hours at room temperature and then labeled with Alexa Fluor 488 Goat Anti-Rabbit IgG Secondary Antibody (Product # A-11008) at a dilution of 1:400 for 30 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor 594 Phalloidin (Product # A12381). Panel d is a merged image showing cytoplasmic localization. Panel e shows no primary antibody control. The images were captured at 20X magnification.

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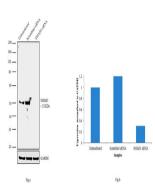
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SMAD3 Antibody (51-1500) in IHC

Immunohistochemical staining of human breast carcinoma using Rb anti-Smad3 (PAD: LPC3) (Product # 51-1500).

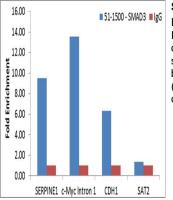
SMAD3 Antibody (51-1500) in WB



Knockdown of SMAD3 was achieved by transfecting A549 with SMAD3 specific siRNAs (Silencer® select Product # s8400). Western blot analysis (Fig. a) was performed using whole cell extracts from the SMAD3 knockdown cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blots were probed with SMAD3 Polyclonal Antibody (Product # 51-1500, 1 µg/mL) and Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Secondary Antibody, HRP conjugate (Product # A27036, 0.25 µg/mL, 1:4000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Absence of signal upon siRNA mediated knock down confirms that antibody is specific to SMAD3.

SMAD3 Antibody (51-1500) in WB

Western blot analysis of SMAD3 (Fig. a) was performed by loading 20 µg of HeLa Control (lane 1), HeLa SMAD3 knockout (lane 2) whole cell extracts. SMAD3 was detected at 50 kDa using SMAD3 polyclonal Antibody (Product # 51-1500, 1µg/mL) and Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Secondary Antibody HRP conjugate (Product # A27036, 1:4000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Loss of signal in CRISPR mediated knockout (KO) confirms that antibody is specific to SMAD3.



SMAD3 Antibody (51-1500) in ChIP

Enrichment of endogenous SMAD3 protein at specific gene loci using Anti-SMAD3 Antibody: Chromatin Immunoprecipitation (ChIP) was performed using Anti-SMAD3 Rabbit Polyclonal Antibody (Product # 51-1500, 4 μg) on sheared chromatin from 2 million MCF7 cells treated with TGFb (7 ng/mL for 1 hour) using the MAGnify ChIP system kit (Product # 49-2024). Normal Rabbit IgG was used as a negative IP control. The purified DNA was analyzed by qPCR with PCR primer pairs over SERPINE1, c-Myc Intron 1 and CDH1 (active) and SAT2 satellite repeats (inactive). Data is presented as fold enrichment of the antibody signal versus the negative control IgG using the comparative CT method.

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8 Immunocytochemistry	References
Species / Dilution	Summary
Human / 1:100	51-1500 was used in immunocytochemistry to examine the association between CEACAM1 isoform balance and hepatocellular carcinoma malignant potential and investigate the molecular interaction between CEACAM1 and beta2SP
	Annals of surgical oncology (2014; 21 Suppl 4: S505) "CEACAM1 long cytoplasmic domain isoform is associated with invasion and recurrence of hepatocellular carcinoma." Author(s):Kiriyama S,Yokoyama S,Ueno M,Hayami S,Ieda J,Yamamoto N,Yamaguchi S,Mitani Y,Nakamura Y,Tani M, Mishra L,Shively JE,Yamaue H PubMed Article URL:http://dx.doi.org/10.1245/s10434-013-3460-1
Luckana / Nat Oitad	The Journal of biological chemistry (2005; 280: 21858)
Luciferase / Not Cited	"Smad7 abrogates transforming growth factor-beta1-mediated growth inhibition in COLO-357 cells through
Human / Not Cited Rat / Not Cited	functional inactivation of the retinoblastoma protein." Author(s):Boyer Arnold N,Korc M PubMed Article URL:http://dx.doi.org/10.1074/jbc.M500583200
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	Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc (2014; 27: 1101) "Improved detection reveals active -papillomavirus infection in skin lesions from kidney transplant recipients." Author(s):Borgogna C,Lanfredini S,Peretti A,De Andrea M,Zavattaro E,Colombo E,Quaglia M,Boldorini R,Miglio U, Doorbar J,Bavinck JN,Quint KD,de Koning MN,Landolfo S,Gariglio M PubMed Article URL:http://dx.doi.org/10.1038/modpathol.2013.240
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	Author(s):Brandsma CA,Timens W,Jonker MR,Rutgers B,Noordhoek JA,Postma DS PubMed Article URL:http://dx.doi.org/10.1152/ajplung.00152.2013
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Mouse / 1:100	American journal of physiology. Lung cellular and molecular physiology (2006; 290: L120) "BMP-7 opposes TGF-beta1-mediated collagen induction in mouse pulmonary myofibroblasts through Id2." Author(s):Izumi N,Mizuguchi S,Inagaki Y,Saika S,Kawada N,Nakajima Y,Inoue K,Suehiro S,Friedman SL,Ikeda K PubMed Article URL:http://dx.doi.org/10.1152/ajplung.00171.2005
Human / Not Cited	The Journal of biological chemistry (2005; 280: 8079) "Ultraviolet irradiation induces Smad7 via induction of transcription factor AP-1 in human skin fibroblasts." Author(s):Quan T,He T,Voorhees JJ,Fisher GJ PubMed Article URL:http://dx.doi.org/10.1074/jbc.M409647200
Human / Not Cited	Breast cancer research : BCR (2004; 6: R215)
Mouse / Not Cited	"Induction by transforming growth factor-beta1 of epithelial to mesenchymal transition is a rare event in Author(s):Brown KA,Aakre ME,Gorska AE,Price JO,Eltom SE,Pietenpol JA,Moses HL PubMed Article URL:http://dx.doi.org/10.1186/bcr778
25 Western Blot Reference	
Species / Dilution	Summary
	51-1500 was used in western blot to investigate the role of nonreceptor tyrosine focal adhesion kinase TGF-beta signaling
Mouse / Not Cited	The Journal of biological chemistry (2011; 286: 17841) "Non-Smad transforming growth factor- signaling regulated by focal adhesion kinase binding the p85 subunit of phosphatidylinositol 3-kinase." Author(s):Hong M,Wilkes MC,Penheiter SG,Gupta SK,Edens M,Leof EB PubMed Article URL:http://dx.doi.org/10.1074/jbc.M111.233676

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51-1500 was used in western blot to report a novel mechanism by which cells become resistant to TGF-beta-mediated growth suppression
Molecular cancer research : MCR (2011; 9: 78) "Distinctive mechanism for sustained TGF- signaling and growth inhibition: MEK1 activation-dependent stabilization of type II TGF- receptors." Author(s):Chen G,Ghosh P,Longo DL PubMed Article URL:http://dx.doi.org/10.1158/1541-7786.MCR-10-0216
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51-1500 was used in western blot to test if inactivation of TGF-beta1 combined with oncogenic activation of K-ras leads to early initiation and faster progression to lung adenocarcinoma
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51-1500 was used in western blot to demonstrate functional roles for Smad3, Hey1, and Jagged1/Notch in mediating TGF-beta-induced epithelial-to-mesenchymal transitions
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"Smad-binding defective mutant of transforming growth factor beta type I receptor enhances tumorigenesis but suppresses metastasis of breast cancer cell lines." Author(s):Tian F,Byfield SD,Parks WT,Stuelten CH,Nemani D,Zhang YE,Roberts AB PubMed Article URL:http://dx.doi.org/10.1158/0008-5472.CAN-04-0030
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Tag / Not Cited Human / Not Cited	"Cellular response to hypoxia involves signaling via Smad proteins." Author(s):Zhang H,Akman HO,Smith EL,Zhao J,Murphy-Ullrich JE,Batuman OA PubMed Article URL:http://dx.doi.org/10.1182/blood-2002-02-0629
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