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Anti-Smad3 Rabbit Monoclonal Antibody

Catalog Number: M00059

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Overview

Product Name	Anti-Smad3 Rabbit Monoclonal Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Smad3 Rabbit Monoclonal Antibody catalog # M00059. Tested in WB, IHC, ICC/IF, Flow Cytometry applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal FG-19
Formulation	Rabbit IgG in stabilizing components, phosphate buffered saline, pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol. *This antibody is supplied in a stabilized formulation. Compatibility with conjugation reactions depends on the chemistry of the conjugation method used. For conjugation methods that are not compatible with the stabilizing components present in this formulation, a carrier-free antibody format is required.
Storage Instructions	Store at -20°C for one year. For short term storage and frequent use, store at 4°C for up to one month. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P84022

Technical Details

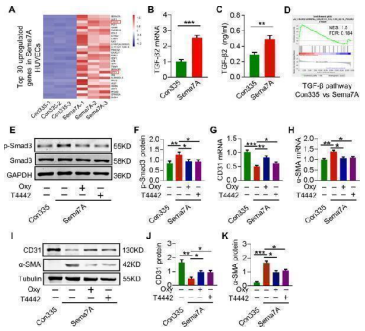
Immunogen	A synthesized peptide derived from human Smad3
Isotype	Rabbit IgG
Form	Liquid
Concentration	0.5mg/ml
Purification	Affinity-chromatography
Suggested Dilutions	WB, 1:500-2000

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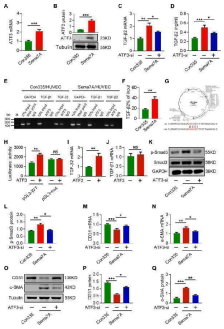
	IHC, 1:50-200 ICC/IF, 1:50-200 FC, 1:50

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Anti-Smad3 Rabbit Monoclonal Antibody (M00059) Images



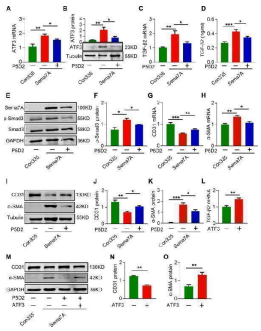
TGF-beta2 gene expression and TGF-Smad signaling are augmented in Sema7A-HUVECs. a The top 30 upregulated genes in Sema7A-HUVECs compared with Con335-HVUECs. b TGF-beta2 mRNA level was analyzed by qPCR normalized to GAPDH. Fold changes are shown. Data are mean ± SEM, N = 3, *** p < 0.001. c The concentration of TGF-beta2 in cell supernatant was detected by ELISA. N = 10. Unpaired two-tailed Student's t tests was used to analysis the data. Data are mean ± SEM, ** p < 0.01. d GSEA based on gene ontology (GO) pathway database showed TGF-beta signaling pathway was enrich in Sema7A-HUVECs. e , f Cells were treated with Oxymatrine (Oxy) (20 umol/l) or T4442 (1 ug/ml) and the lysates were analyzed by western blotting for Smad3 phosphorylation, normalized to total Smad3. Data are mean ± SEM, N = 3, * p < 0.05; ** p < 0.01. g , h CD31 and alpha-SMA mRNA in cells treated with inhibitors were analyzed by qPCR normalized to GAPDH. Fold changes are shown. Data are mean ± SEM, N = 3, * p < 0.05; ** p < 0.01; *** p < 0.001. i – k CD31 and alpha-SMA proteins in cells treated with inhibitors were analysis by western blotting, normalized to tubulin. Data are mean ± SEM, N = 3, * p < 0.05; ** p < 0.01; *** p < 0.001. T4442: TGF-beta2 blocking antibody; Oxymatrine (Oxy): TGF/Smad signaling pathway inhibitor. Index in PubMed under a CC BY license. PMID: 32826874



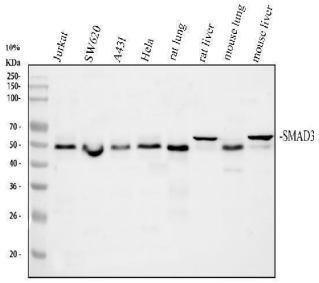
Inhibition of ATF3 reduced TGF-beta2 expression and Sema7A-induced EndMT. a ATF3 mRNA level was analyzed by qPCR normalized to GAPDH. Fold changes are shown. Data are mean ± SEM, N = 3, *** p < 0.001 b ATF3 protein expression were analyzed by western blotting, normalized to tubulin. Data are mean ± SEM, N = 3, *** p < 0.001. c TGF-beta2 mRNA level was analyzed by qPCR normalized to GAPDH. Fold changes are shown. Data are mean ± SEM, N = 3, * p < 0.05; ** p < 0.01. d The concentration of TGF-beta2 in cell supernatant was detected by ELISA among Con335-HUVECs, Sema7A-HUVECs, and Sema7A-HUVECs + ATF3-siRNA. N = 10. Data are mean ± SEM, * p < 0.05; *** p < 0.001. e Chip-qPCR product in agarose gel electrophoresis. f Chip-qPCR TGF-beta2 percentage of input in con335-HUVECs and Sema7A - HUVECs were analyzed by qPCR normalized to IgG. Data are mean ± SEM, N = 3, ** p < 0.01. g Schematic graph of the constructed reporter plasmid. TGF-beta2 mut indicates the TGF-beta2 mutation promoter region in ATF3 binding site. The mutated nucleotides in TGF-beta2 fragments are in red letters. h Luciferase reporter assays were performed on HEK 293 T cells. Data are mean ± SEM, N = 3, ** p < 0.01 vs negative control. i , j ATF3-overexpression plasmid was transfected to HUVECs, and the mRNA levels of TGF - beta2 and TGF-beta1 were performed by qPCR normalized to GAPDH. Fold changes are shown. Data are mean ± SEM, N = 3, ** p < 0.01. k , l P-Smad3 protein in cells treated with siRNA was analyzed by Western blotting, normalized to total Smad3. Data are mean ± SEM, N

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= 3, * p < 0.05; ** p < 0.01. m – q CD31 and alpha-SMA RNA and proteins in cells treated with siRNA or control was analyzed by qPCR and western blotting. Data are mean ± SEM, N = 3, * p < 0.05; ** p < 0.01; *** p < 0.001. Index in PubMed under a CC BY license. PMID: 32826874



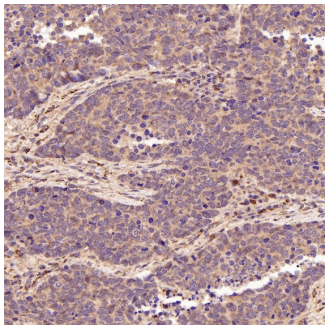
B1 integrin mediates Sema7A signal to TGF-beta2 via ATF3. a Cells were treated with beta1 integrin antibody (P5D2), and ATF3 mRNA level was analyzed by qPCR normalized to GAPDH. Fold changes are shown. Data are mean ± SEM, N = 3, * p < 0.05; ** p < 0.0.1. b ATF3 protein expression was analyzed by western blotting, normalized to tubulin. Data are mean ± SEM, N = 3, * p < 0.05; ** p < 0.01. c TGF-beta2 mRNA level was analyzed by qPCR normalized to GAPDH. Fold changes are shown. Data are mean ± SEM, N = 3, * p < 0.05; ** p < 0.0.1. d The concentration of TGF-beta2 in cell supernatant was detected by ELISA for Con335-HUVECs, Sema7A-HUVECs, and Sema7A-HUVECs + P5D2. Data are mean ± SEM, N = 10, * p < 0.05; *** p < 0.001. e , f Smad3 phosphorylation were analyzed by western blotting, normalized to total Smad3. Data are mean ± SEM, N = 3, * p < 0.05. g , h CD31 and alpha-SMA mRNA level were analyzed by qPCR normalized to GAPDH. Fold changes are shown. Data are mean ± SEM, N = 3, * p < 0.05; ** p < 0.01; *** p < 0.001. i – k CD31 and alpha-SMA proteins were analyzed by western blotting, normalized to tubulin. Data are mean ± SEM, N = 3, * p < 0.05; ** p < 0.01; *** p < 0.001. l ATF3 overexpression plasmid was transfected into P5D2 incubated Sema7A-HUVECs, and TGF-beta2 mRNA expression in Sema7A-HUVECs + P5D2 and Sema7A-HUVECs + P5D2 + ATF3 were analyzed by qPCR normalized to GAPDH. Fold changes are shown. Data are mean ± SEM, N = 3, ** p < 0.01. m – o CD31 and alpha-SMA protein expressions were analyzed by western blotting, normalized to GAPDH. Data are mean ± SEM, N = 3, ** p < 0.01 (Sema7A-HUVECs + P5D2 vs Sema7A-HUVECs + P5D2 + ATF3). P5D2 beta1 integrin antibody. Index in PubMed under a CC BY license. PMID: 32826874



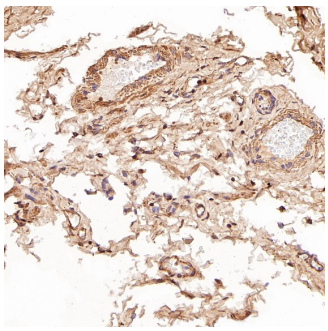
Western blot analysis of Smad3 using anti-Smad3 antibody (M00059). Electrophoresis was performed on a 10% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human Jurkat whole cell lysates, Lane 2: human SW620 whole cell lysates, Lane 3: human A431 whole cell lysates, Lane 4: human Hela whole cell lysates, Lane 5: rat lung tissue lysates, Lane 6: rat liver tissue lysates, Lane 7: mouse lung tissue lysates, Lane 7: mouse liver tissue lysates.After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-Smad3 antigen affinity purified monoclonal antibody (M00059) at 1:1000 overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate

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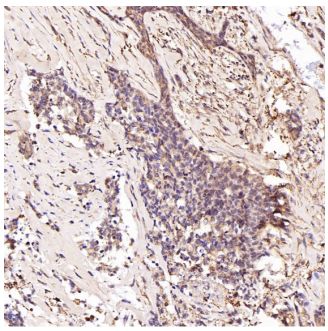
(Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for Smad3 at approximately 50 kDa. The expected band size for Smad3 is at 48 kDa.



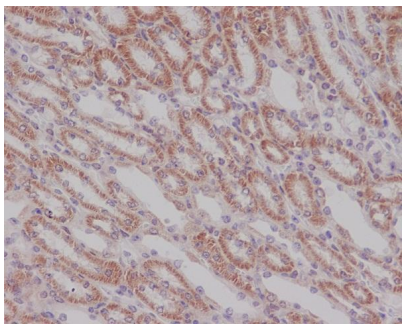
Immunohistochemical analysis of paraffin-embedded Human lung large cell cancer, using the Antibody at 1:500 dilution.



Immunohistochemical analysis of paraffin-embedded Human testis cancer, using the Antibody at 1:500 dilution.



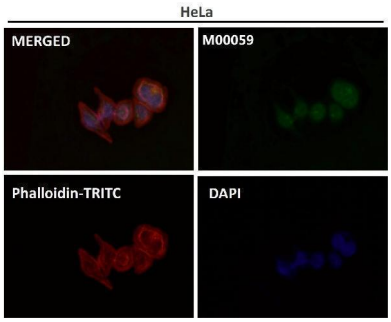
Immunohistochemical analysis of paraffin-embedded Human breast cancer, using the Antibody at 1:500 dilution.

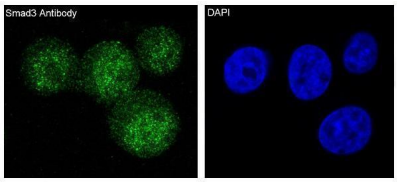


Immunohistochemical analysis of paraffin-embedded mouse kidney, using Smad3 Antibody.

Immunofluorescent analysis using the Antibody at 1:150 dilution.

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Immunofluorescent analysis of Hela cells, using Smad3 Antibody.

2 Publications Citing This Product

1. PubMed ID: 32826874, Hong L,Li F,Tang C,Li L,Sun L,Li X,Zhu L.Semaphorin 7A promotes endothelial to mesenchymal transition through ATF3 mediated TGF-beta2/Smad signaling.Cell Death Dis.2020 Aug 10;11(8):695.doi:10.1038/s41419-020-02818-x.PMID:32826874;PMCID:PMC7442651.

2. PubMed ID: 25356121, Shi K, Jiang J, Ma T, Xie J, Duan L, Chen R, Song P, Yu Z, Liu C, Zhu Q, Zheng J. Int J Clin Exp Med. 2014 Sep 15;7(9):2645-50. Ecollection 2014. Dexamethasone Attenuates Bleomycin-Induced Lung Fibrosis In Mice Through Tgf-??, Smad3 And Jak-Stat P...

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