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Anti-Phospho-SMAD3 S423/S425 Antibody

Catalog Number: P00059

About SMAD3

This antibody 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-b superfamily. Briefly, activated type I receptor phosphorylates receptor-activated Smads (R-Smads) at their c-terminal two extreme serines in the SSXS motif, e.g. Smad2 and Smad3 proteins in the TGF-b pathway, or Smad1, Smad5 or Smad8 in the BMP pathway. Then the phosphorylated R-Smad 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.

Overview

Product Name	Anti-Phospho-SMAD3 S423/S425 Antibody
Reactive Species	Human
Description	Boster Bio Anti-Phospho-SMAD3 S423/S425 Antibody (Catalog # P00059). Tested in ELISA, IHC, WB applications. This antibody reacts with Human.
Application	ELISA, Flow Cytometry, IHC, WB
Clonality	Polyclonal
Formulation	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2, 0.01% (w/v) Sodium Azide
Storage Instructions	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 date is one (1) year from date of opening. (Ship on dry ice.)
Host	Rabbit
Uniprot ID	P84022

Technical Details

Immunogen	Anti-SMAD3 pS423pS425 antibody was prepared from whole rabbit serum produced by repeated immunizations with a dual phosphorylated synthetic peptide corresponding to a c-terminal region with Serine 423 and Serine 425 of human SMAD3 protein.
Predicted Reactive Species	Bovine, Chicken, Xenopus Laevis, Xenopus Tropicalis, Zebrafish
Cross Reactivity	No cross reactivity with other proteins.
Isotype	IgG

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Form	Liquid (sterile filtered)
Concentration	1.16 mg/ml by UV absorbance at 280 nm
Purification	This affinity-purified antibody is directed against the phosphorylated form of human Smad3 protein at the pS423 and pS425 residues. 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 pS423 and pS425 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 Smad1 and Smad5. Negligible reactivity is seen against other phosphorylated Smad family members. A BLAST analysis was used to suggest cross-reactivity with Smad3 from human, Xenopus laevis, Xenopus tropicalis, zebrafish, rat, mouse, swine, bovine and chicken based on 100% sequence homology with the immunogen. Reactivity against homologues from other sources is not known.
Suggested Dilutions	ELISA: 1:15,000 - 1:30,000 Flow Cytometry: User optimized IHC: 1:500 WB: 1:2,000 - 1:20,000 This affinity purified antibody has been tested for use in ELISA, immunohistochemistry, and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 48 kDa in size corresponding to phosphorylated Smad3 protein by western blotting in the appropriate stimulated tissue or cell lysate or extract. Less than 0.2% reactivity is observed against the non-phosphorylated form of the immunizing peptide. This antibody is phospho specific for dual phosphorylated pS423 and pS425 of Smad3. Stimulation with 2 ng/ml TGF-beta for 1 hour is suggested.

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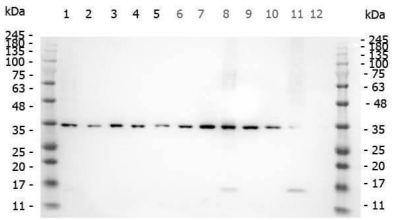
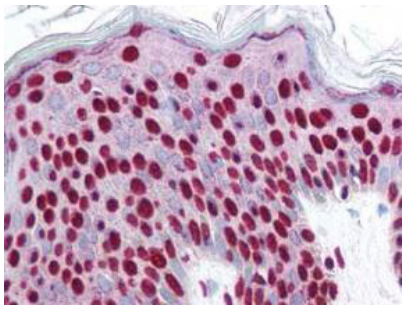
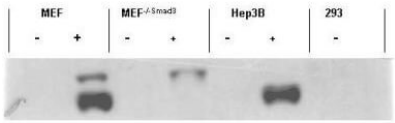
Anti-Phospho-SMAD3 S423/S425 Antibody (P00059) Images



Western Blot of Rabbit anti-SMAD3 pS423 pS425 antibody. Marker: Opal Pre-stained ladder . Lane 1: HEK293 lysate . Lane 2: HeLa Lysate . Lane 3: MCF-7 Lysate . Lane 4: Jurkat Lysate . Lane 5: A431 Lysate . Lane 6: A549 Lysate . Lane 7: LNCap Lysate . Lane 8: MOLT-4 Lysate . Lane 9: Ramos Lysate . Lane 10: Raji Lysate . Lane 11: A-172 Lysate . Lane 12: NIH/3T3 Lysate . Load: 10 µg per lane. Primary antibody: SMAD3 pS423 pS425antibody at 1ug/mL overnight at 4C. Secondary antibody: Peroxidase rabbit secondary antibody at 1:30,000 for 60 min at RT. Blocking Buffer: 1% Casein-TTBS for 30 min at RT. Predicted/Observed size: 35 kDa for SMAD3 pS423 pS425.



Boster's affinity purified anti-Smad3 pS423 pS425 antibody was used at 2.5 ug/ml to detect signal in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows strong nuclear staining in the majority of epidermal keratinocytes at 40X. Tissue was formalin-fixed and paraffin embedded. The image shows localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. Personal Communi-cation, Tina Roush, LifeSpanBiosciences, Seattle, WA.



Western blot using Boster's affinity purified anti-Smad3 pS423 pS425 antibody shows detection of endogenous Smad3 in stimulated cell lysates. Lysates were prepared from control cells (- lanes), or cells stimulated with 2 ng/ml TGF (+lanes) for 1 hour. This reagent recognizes phosphorylated Smad3 and has negligible reactivity against non-phosphorylated Smad3 protein. Personal Communication. Ying Zhang, NIH, CCR, Bethesda, MD.

4 Publications Citing This Product

1. PubMed ID: 34093874, Wang Q,Liu J,Hu Y,Pan T,Xu Y,Yu J,Xiong W,Zhou Q,Wang Y.Local administration of liposomal-based Srpx2 gene therapy reverses pulmonary fibrosis by blockading fibroblast-to-myofibroblast transition.Theranostics.2021 May 13;11(14):7110-7125.doi:10.7150/thno.61085. PMID:34093874;PMCID:PMC8171094.

2. 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.

3. PubMed ID: 25820389, Xu T, Ni Mm, Huang C, Meng Xm, He Yh, Zhang L, Li J. Inflammation. 2015 Oct;38(5):1794-804. Doi: 10.1007/S10753-015-0157-6. Nlrc5 Mediates Il-6 And Il-1?? Secretion In Lx-2 Cells And Modulated By The Nf-??b/Smad3 Pathway.

Visit bosterbio.com/anti-smad3-phospho-s423-phospho-s425-antibody-p00059-boster.html to see all 4 publications.

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