

Anti-SMAD2 Antibody

Catalog Number: A00090-1

About SMAD2

Smad2 (Mothers against decapentaplegic homolog 2), also known as MADR2, MADH2, SMAD family member 2 or SMAD2, is a protein that in humans is encoded by the SMAD2 gene. MAD homolog 2 belongs to the SMAD, a family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (Mad) and the C. elegans gene Sma. Eppert et al. mapped the MADR2 gene close to DPC4 at 18q21, a region which is frequently deleted in colorectal cancers. Riggins et al. mapped the human MADH2 gene to 18q21. Nakao et al. refined the localization of the SMAD2 gene to 18q21.1, approximately 3 Mb proximal to DPC4, by fluorescence in situ hybridization. SMAD2 mediates the signal of the transforming growth factor (TGF)-beta, and thus regulates multiple cellular processes, such as cell proliferation, apoptosis, and differentiation. This protein is recruited to the TGF-beta receptors through its interaction with the SMAD anchor for receptor activation (SARA) protein. In response to TGF-beta signal, this protein is phosphorylated by the TGF-beta receptors.

Overview

Product Name	Anti-SMAD2 Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-SMAD2 Antibody catalog # A00090-1. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q15796

Technical Details

Immunogen	E.coli-derived human SMAD2 recombinant protein (Position: E83-Q264).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-14) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized

Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.5ug/ml</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml</p> <p>Immunocytochemistry/Immunofluorescence, 5ug/ml</p> <p>Flow Cytometry (Fixed), 1-3ug/1x10⁶ cells</p> <p>ELISA, 0.1-0.5ug/ml</p>

Anti-SMAD2 Antibody (A00090-1) Images

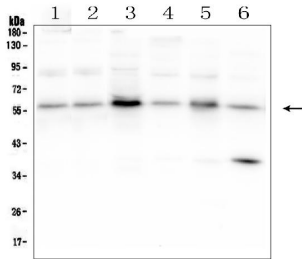


Figure 1. Western blot analysis of SMAD2 using anti-SMAD2 antibody (A00090-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human placenta tissue lysates

Lane 2: rat liver tissue lysates

Lane 3: mouse testicular tissue lysates

Lane 4: mouse heart tissue lysates

Lane 5: mouse lung tissue lysates

Lane 6: mouse lung tissue lysates

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA 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-SMAD2 antigen affinity purified polyclonal antibody (Catalog # A00090-1) at 0.5 ug/mL 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 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for SMAD2 at approximately 60KD. The expected band size for SMAD2 is at 52KD.

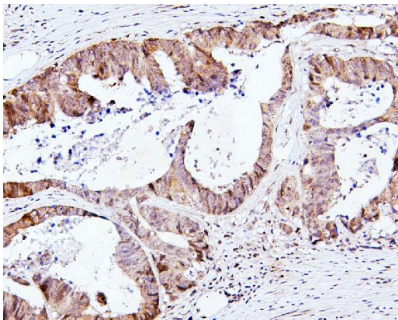


Figure 2. IHC analysis of SMAD2 using anti-SMAD2 antibody (A00090-1).

SMAD2 was detected in paraffin-embedded section of human colon cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-SMAD2 Antibody (A00090-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

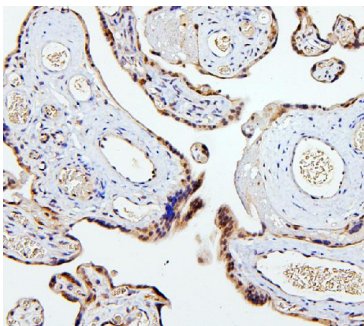


Figure 3. IHC analysis of SMAD2 using anti-SMAD2 antibody (A00090-1).

SMAD2 was detected in paraffin-embedded section of human placenta tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-SMAD2 Antibody (A00090-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

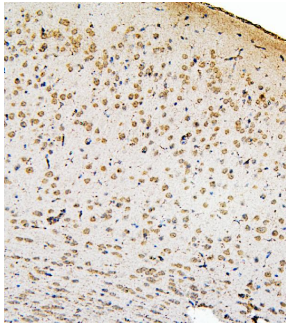


Figure 4. IHC analysis of SMAD2 using anti-SMAD2 antibody (A00090-1). SMAD2 was detected in paraffin-embedded section of mouse brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-SMAD2 Antibody (A00090-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

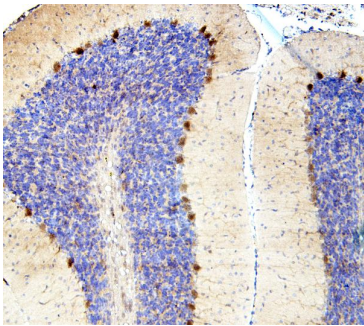


Figure 5. IHC analysis of SMAD2 using anti-SMAD2 antibody (A00090-1). SMAD2 was detected in paraffin-embedded section of rat brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-SMAD2 Antibody (A00090-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

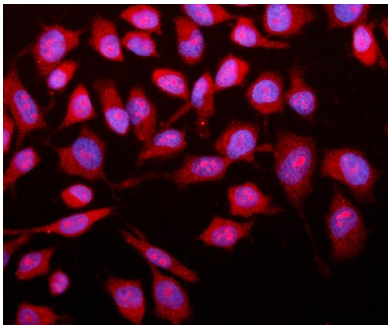


Figure 6. IF analysis of SMAD2 using anti-SMAD2 antibody (A00090-1). SMAD2 was detected in immunocytochemical section of HELA cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5ug/mL rabbit anti-SMAD2 Antibody (A00090-1) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Rabbit IgG (BA1142) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

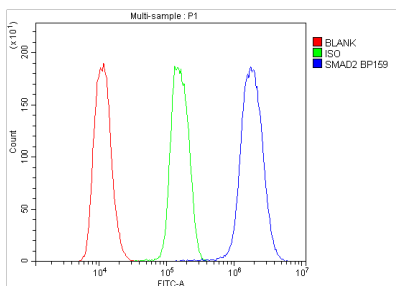


Figure 7. Flow Cytometry analysis of K562 cells using anti-SMAD2 antibody (A00090-1). Overlay histogram showing K562 cells stained with A00090-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-SMAD2 Antibody (A00090-1, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

1. PubMed ID: 10.1155/2016/2601830, Effect of Kuijie Granule on the Expression of TGF-beta/Smads Signaling Pathway in Patients with Ulcerative Colitis
2. PubMed ID: 26261569, Astragaloside effect on TGF- β 1, SMAD2/3, and β 1-SMA expression in the kidney tissues of diabetic KKAY mice
3. PubMed ID: 30090338, Mouse hepatic neoplasm formation induced by trace level and low frequency exposure to diethylnitrosamine through β -catenin signaling pathway

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