

Anti-SMAD2 Antibody Picoband™ (monoclonal, 3C4)

Catalog Number: M00090-3

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 Picoband™ (monoclonal, 3C4)
Reactive Species	Human
Description	Boster Bio Anti-SMAD2 Antibody Picoband™ (monoclonal, 3C4) catalog # M00090-3. Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IHC, WB
Clonality	Monoclonal 3C4
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
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	Mouse
Uniprot ID	Q15796

Technical Details

Immunogen	E.coli-derived human SMAD2 recombinant protein (Position: E83-Q264).
Predicted Reactive Species	Hepatitis Virus
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG1

Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.25-0.5ug/ml, Human Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human Flow Cytometry, 1-3ug/1x10⁶ cells, Human

Anti-SMAD2 Antibody Picoband™ (monoclonal, 3C4) (M00090-3) Images

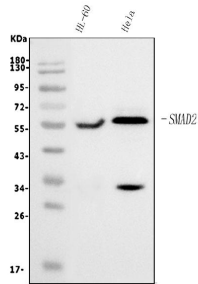


Figure 1. Western blot analysis of SMAD2 using anti-SMAD2 antibody (M00090-3).

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 30ug of sample under reducing conditions.

Lane 1: human HL-60 whole cell lysates,

Lane 2: human Hela whole cell 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 mouse anti-SMAD2 antigen affinity purified monoclonal antibody (Catalog # M00090-3) 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-mouse 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 # EK1001) with Tanon 5200 system. A specific band was detected for SMAD2 at approximately 55KD. The expected band size for SMAD2 is at 55KD.

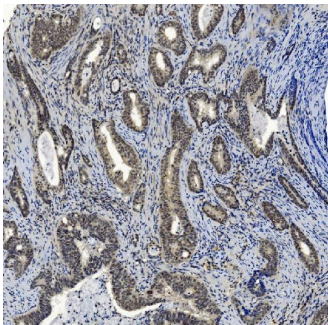


Figure 2. IHC analysis of SMAD2 using anti-SMAD2 antibody (M00090-3).

SMAD2 was detected in paraffin-embedded section of human gallbladder adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-SMAD2 Antibody (M00090-3) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

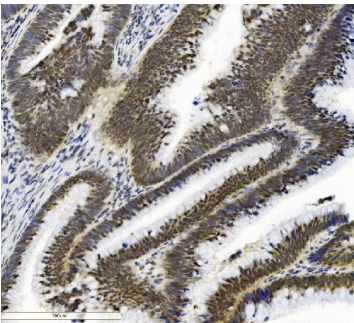
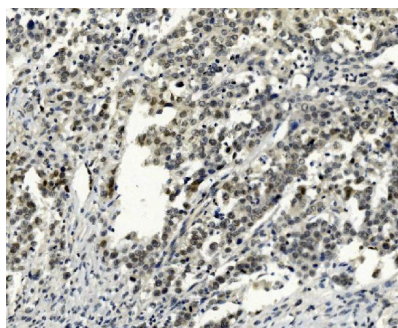


Figure 3. IHC analysis of SMAD2 using anti-SMAD2 antibody (M00090-3).

SMAD2 was detected in paraffin-embedded section of human low-differentiated adenocarcinoma of the intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-SMAD2 Antibody (M00090-3) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

Figure 4. IHC analysis of SMAD2 using anti-SMAD2 antibody



(M00090-3). SMAD2 was detected in paraffin-embedded section of human pancreatic cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-SMAD2 Antibody (M00090-3) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

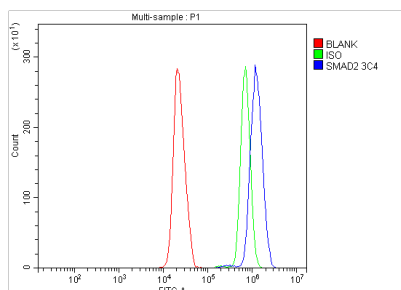


Figure 5. Flow Cytometry analysis of HL-60 cells using anti-SMAD2 antibody (M00090-3). Overlay histogram showing HL-60 cells stained with M00090-3 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-SMAD2 Antibody (M00090-3, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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