

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

Catalog # ABO15030

Product Information

Application	WB, IHC, FC
Primary Accession	Q15796
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-SMAD2 Antibody Picoband™ (monoclonal, 3C4) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	4087
Other Names	Mothers against decapentaplegic homolog 2, MAD homolog 2, Mothers against DPP homolog 2, JV18-1, Mad-related protein 2, hMAD-2, SMAD family member 2, SMAD 2, Smad2, hSMAD2, SMAD2, MADH2, MADR2
Calculated MW	52306
Application Details	Western blot, 0.25-0.5 µg/ml, Human Immunohistochemistry (Paraffin-embedded Section), 2-5 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na ₂ HPO ₄ .
Clone Names	Clone: 3C4
Immunogen	E.coli-derived human SMAD2 recombinant protein (Position: E83-Q264).
Purification	Immunogen affinity purified.
Storage	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.

Protein Information

Name	SMAD2 (HGNC:6768)
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Synonyms

MADH2, MADR2

Function

Receptor-regulated SMAD (R-SMAD) that is an intracellular signal transducer and transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinases. Binds the TRE element in the promoter region of many genes that are regulated by TGF-beta and, on formation of the SMAD2/SMAD4 complex, activates transcription. Promotes TGF-beta-mediated transcription of odontoblastic differentiation genes in dental papilla cells (By similarity). Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator. May act as a tumor suppressor in colorectal carcinoma (PubMed:[8752209](#)).

Cellular Location

Cytoplasm. Nucleus Note=Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4 or with IPO7 (PubMed:21145499, PubMed:9865696). On dephosphorylation by phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1 (PubMed:16751101, PubMed:19289081). Localized mainly to the nucleus in the early stages of embryo development with expression becoming evident in the cytoplasm at the blastocyst and epiblast stages (By similarity) {ECO:0000250|UniProtKB:Q62432, ECO:0000269|PubMed:16751101, ECO:0000269|PubMed:19289081, ECO:0000269|PubMed:21145499, ECO:0000269|PubMed:9865696}

Tissue Location

Expressed at high levels in skeletal muscle, endothelial cells, heart and placenta.

Background

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.

Images

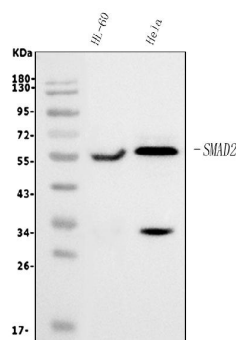


Figure 1. Western blot analysis of SMAD2 using anti-SMAD2 antibody (M00090-3).

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 µg/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.

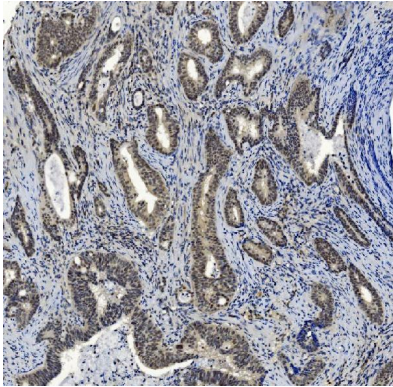


Figure 2. IHC analysis of SMAD2 using anti-SMAD2 antibody (M00090-3). SMAD2 was detected in paraffin-embedded section of human gallbladder adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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.

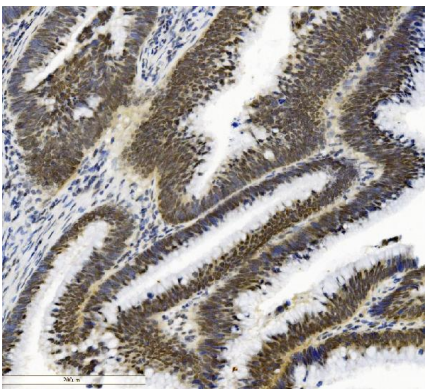


Figure 3. IHC analysis of SMAD2 using anti-SMAD2 antibody (M00090-3). SMAD2 was detected in paraffin-embedded section of human low-differentiated adenocarcinoma of the intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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.

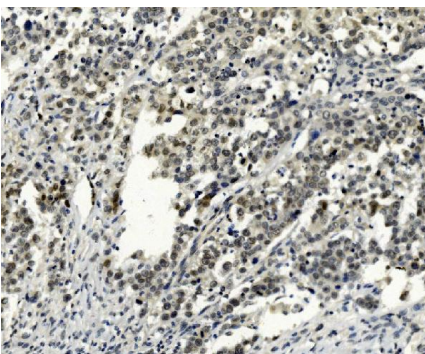
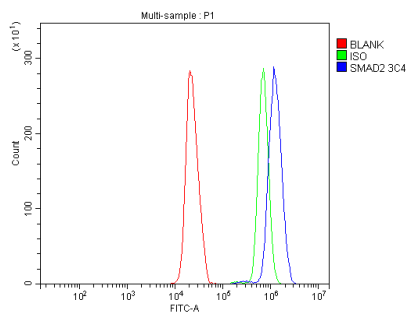


Figure 4. IHC analysis of SMAD2 using anti-SMAD2 antibody (M00090-3). SMAD2 was detected in paraffin-embedded section of human pancreatic cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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, 1 µg/1x10⁶ cells) for 30



min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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