

Anti-SMAD5 Antibody Picoband™

Catalog Number: A01423

About SMAD5

Mothers against decapentaplegic homolog 5 also known as SMAD5 is a protein that in humans is encoded by the SMAD5 gene. It is mapped to 5q31.1. The protein encoded by this gene is involved in the transforming growth factor beta signaling pathway that results in an inhibition of the proliferation of hematopoietic progenitor cells. The encoded protein is activated by bone morphogenetic proteins type 1 receptor kinase, and may be involved in cancer. Alternative splicing results in multiple transcript variants.

Overview

Product Name	Anti-SMAD5 Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-SMAD5 Antibody Picoband™ catalog # A01423. Tested in IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
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	Q99717

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence in the middle region of human SMAD5, which shares 95.5% amino acid (aa) sequence identity with both mouse and rat SMAD5.
Predicted Reactive Species	Human
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 (SV0002-1) 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, 0.25-0.5ug/ml, Human, Mouse, Rat</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Rat</p> <p>Immunocytochemistry/Immunofluorescence, 2ug/ml, Human</p>

Anti-SMAD5 Antibody Picoband™ (A01423) Images

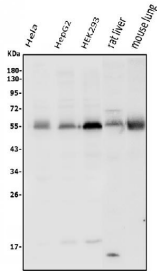


Figure 1. Western blot analysis of SMAD5 using anti-SMAD5 antibody (A01423).

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 Hela whole cell lysates,

Lane 2: human HepG2 whole cell lysates,

Lane 3: human HEK293 whole cell lysates,

Lane 4: rat liver tissue lysates,

Lane 5: 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-SMAD5 antigen affinity purified polyclonal antibody (Catalog # A01423) 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 SMAD5 at approximately 52KD. The expected band size for SMAD5 is at 52KD.

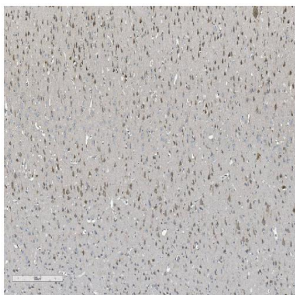


Figure 2. IHC analysis of SMAD5 using anti-SMAD5 antibody (A01423).

SMAD5 was detected in paraffin-embedded section of rat brain 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 1ug/ml rabbit anti-SMAD5 Antibody (A01423) 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.

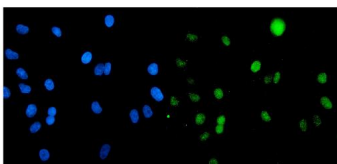


Figure 3. IF analysis of SMAD5 using anti-SMAD5 antibody (A01423).

SMAD5 was detected in immunocytochemical section of A549 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 2ug/mL rabbit anti-SMAD5 Antibody (A01423) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) 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.

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