Phospho-Smad1/5/9 (S463/465/467) Antibody [K12G16]

Catalog No.C06418 Batch:C0641803

Product Datasheet

Biological Description

Specificity	Phospho-Smad1/5/9 (S463/465/467) Antibody [K12G16] recognizes endogenous levels of total Phospho-SMAD1 + SMAD5 + SMAD9 when phosphorylated at Ser 463 + Ser 465 + Ser 467.
Background	Small Body Size (SMA) and Mothers Against Decapentaplegic family 1 (SMAD1), also known as JV4-1, MADH1, or MADR1, is a gene located on human chromosome 5q4. Initially identified during studies on genes associated with breast cancer, SMAD1 has since been recognized as a crucial regulator in tumor progression. It serves as a key mediator of TGF-β signaling and plays an essential role in processes such as cell growth, apoptosis, development, and immune responses. SMAD1 is implicated in the advancement of various malignancies, acting as a transducer of signals from bone morphogenetic proteins (BMPs), which influence a wide range of cellular functions, including proliferation, apoptosis, development, and immune regulation. BMP ligands activate SMAD1 through phosphorylation by BMP receptor kinases. Once phosphorylated, SMAD1 forms a complex with SMAD4, which migrates to the nucleus to regulate gene transcription in conjunction with specific transcription factors. SMAD1 has been shown to facilitate cell invasion and metastasis in multiple cancer types. For example, its overexpression promotes the proliferation of stomach cancer cells in response to BMP-7 and enhances ovarian cancer cell growth upon activation by BMP-9. BMP receptors, part of the TGF-β superfamily of Ser/Thr kinase receptors, play a central role in this signaling cascade. Ligand binding triggers receptor multimerization, autophosphorylation, and activation. The activated receptors then phosphorylate SMAD1 at specific serine residues (Ser463 and Ser465) within the SSXS motif in its carboxy-terminal region. Similar phosphorylation occurs in SMAD5 and SMAD9 (also known as SMAD8). These phosphorylated SMAD proteins subsequently form dimers with the coactivator SMAD4 and translocate to the nucleus, where they regulate the transcription of target genes.

Usage Information

Application	WB, IHC	**Dilution**	WB: 1:1000-1:10000; IHC: 1:100 - 1:800
Reactivity	Human, Mouse, Rat		
Source	Rabbit Monoclonal Antibody	**MW**	58 kDa
Storage Buffer	PBS, pH 7.2+50% Glycerol+0.05% BSA+0.01% NaN_3		
Storage (from the date of receipt)	-20°C (avoid freeze-thaw cycles), 2 years		

Experimental Methods

WB

Experimental Protocol:

Sample preparation
1. Tissue: Lyse the tissue sample by adding an appropriate volume of ice-cold **RIPA/NP-40 Lysis Buffer (containing Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail)**, and homogenize the tissue at a low temperature or lyse it by sonication on ice, then incubate on ice for 30 minutes.
2. Adherent cell: Aspirate the culture medium and transfer the cells into an EP tube. Wash the cells with ice-cold PBS twice. Add an appropriate volume of **RIPA/NP-40 Lysis Buffer (containing Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail)**, sonicate to lyse the cells, and incubate on ice for 30 minutes.
3. Suspension cell: Transfer the culture medium to a pre-cooled centrifuge tube. Centrifuge and aspirate the supernatant. Wash the cells with ice-cold PBS twice.Add an appropriate volume of **RIPA/NP-40 Lysis Buffer (containing Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail)**, sonicate to lyse the cells, and incubate on ice for 30 minutes.
4. Place the lysate into a pre-cooled microcentrifuge tube. Centrifuge at 4°C for 15 min. Collect the supernatant;
5. Remove a small volume of lysate to determine the protein concentration;
6. Combine the lysate with protein loading buffer. Boil 20 μL sample under 95-100°C for 5 min. Centrifuge for 5 min after cool down on ice.

Electrophoretic separation
1. According to the concentration of extracted protein, load appropriate amount of protein sample and marker onto SDS-PAGE gels for electrophoresis. Recommended separating gel (lower gel) concentration: 10%. Reference Table for Selecting SDS-PAGE Separation Gel Concentrations
2. Power up 80V for 30 minutes. Then the power supply is adjusted (110 V~150 V), the Marker is observed, and the electrophoresis can be stopped when the indicator band of the predyed protein Marker where the protein is located is properly separated. (Note that the current should not be too large when electrophoresis, too large current (more than 150 mA) will cause the temperature to rise, affecting the result of running glue. If high currents cannot be avoided, an ice bath can be used to cool the bath.)

Transfer membrane
1. Take out the converter, soak the clip and consumables in the pre-cooled converter;
2. Activate PVDF membrane with methanol for 1 min and rinse with transfer buffer;
3. Install it in the order of "black edge of clip - sponge - filter paper - filter paper - glue -PVDF membrane - filter paper - filter paper - sponge - white edge of clip";
4. The protein was electrotransferred to PVDF membrane. (0.45 μm PVDF membrane is recommended) Reference Table for Selecting PVDF Membrane Pore Size Specifications
Recommended conditions for wet transfer: **200 mA, 120 min**.
(Note that the transfer conditions can be adjusted according to the protein size. For high-molecular-weight proteins, a higher current and longer transfer time are recommended. However, ensure that the transfer tank remains at a low temperature to prevent gel melting.)

Block
1. After electrotransfer, wash the film with TBST at room temperature for 5 minutes;
2. Incubate the film in the blocking solution **(recommending 5% BSA solution)** for 1 hour at room temperature;
3. Wash the film with TBST for 3 times, 5 minutes each time.

Antibody incubation
1. Use 5% skim milk powder to prepare the primary antibody working liquid (recommended dilution ratio for primary antibody 1:1000), gently shake and incubate with the film at 4°C overnight;
2. Wash the film with TBST 3 times, 5 minutes each time;
3. Add the secondary antibody to the blocking solution and incubate with the film gently at room temperature for 1 hour;
4. After incubation, wash the film with TBST 3 times for 5 minutes each time.

Antibody staining
1320. Add the prepared ECL luminescent substrate (or select other color developing substrate according to the second antibody) and mix evenly;
2. Incubate with the film for 1 minute, remove excess substrate (keep the film moist), wrap with plastic film, and expose in the imaging system.

IHC

Experimental Protocol:

Deparaffinization/Rehydration
1. Deparaffinize/hydrate sections:
2. Incubate sections in three washes of xylene for 5 min each.

Experimental Methods

3. Incubate sections in two washes of 100% ethanol for 10 min each.
4. Incubate sections in two washes of 95% ethanol for 10 min each.
5. Wash sections two times in dH2O for 5 min each.
6.Antigen retrieval: For Citrate: Heat slides in a microwave submersed in 1X citrate unmasking solution until boiling is initiated; continue with 10 min at a sub-boiling temperature (95°-98°C). Cool slides on bench top for 30 min.

Staining
1. Wash sections in dH2O three times for 5 min each.
2. Incubate sections in 3% hydrogen peroxide for 10 min.
3. Wash sections in dH2O two times for 5 min each.
4. Wash sections in wash buffer for 5 min.
5. Block each section with 100–400 µl of blocking solution for 1 hr at room temperature.
6. Remove blocking solution and add 100–400 µl primary antibody diluent in to each section. Incubate overnight at 4°C.
7. Remove antibody solution and wash sections with wash buffer three times for 5 min each.
8. Cover section with 1–3 drops HRPas needed. Incubate in a humidified chamber for 30 min at room temperature.
9. Wash sections three times with wash buffer for 5 min each.
10. Add DAB Chromogen Concentrate to DAB Diluent and mix well before use.
11. Apply 100–400 µl DAB to each section and monitor closely. 1–10 min generally provides an acceptable staining intensity.
12. Immerse slides in dH2O.
13. If desired, counterstain sections with hematoxylin.
14. Wash sections in dH2O two times for 5 min each.
15. Dehydrate sections: Incubate sections in 95% ethanol two times for 10 sec each; Repeat in 100% ethanol, incubating sections two times for 10 sec each; Repeat in xylene, incubating sections two times for 10 sec each.
16. Mount sections with coverslips and mounting medium.

References

https://pubmed.ncbi.nlm.nih.gov/34134766/ https://pubmed.ncbi.nlm.nih.gov/9716398/

Application Data

WB

Validated by Capsid Bio



Lane 1: HeLa
Lane 2: HeLa (BMP-4 treated)
Lane 3: HeLa (BMP-4 and phosphatase treated)
Lane 4: Mouse brain
Lane 5: Rat brain