Phospho-Smad1/5/9 (S463/465, S463/465, S465/467) Antibody [L11J17]

Catalog No.C05126 Batch:C0512603

Product Datasheet

Biological Description

Specificity	Phospho-Smad1/5/9 (S463/465, S463/465, S465/467) Antibody [L11J17] recognizes endogenous levels of SMAD1 and SMAD5 protein when phosphorylated at Ser463/465 and SMAD9 (SMAD8) protein when phosphorylated at Ser465/467.
Background	Phospho-Smad1 (Ser 463/465), Smad5 (Ser 463/465), and Smad9 (Ser 465/467) are members of the Smad family of intracellular mediators in the TGF-β and BMP signaling pathways, where they regulate gene expression involved in development, differentiation, and apoptosis. Structurally, they contain an N-terminal MH1 domain for DNA binding, a C-terminal MH2 domain for protein interactions, and a linker region with phosphorylation sites. These Smads are phosphorylated at specific serine residues (Ser 463/465 in Smad1/5 and Ser 465/467 in Smad9) by BMP receptors, which are Ser/Thr kinases. Upon phosphorylation, they dimerize with Smad4 and translocate to the nucleus to regulate target genes, impacting processes like bone formation (Smad1), hematopoiesis (Smad5), and possibly overlapping BMP functions (Smad9). Their expression varies: Smad1 is present in tissues like the brain, liver, and kidney; Smad5 in the brain and muscle; and Smad9 in several tissues but is less characterized.

Usage Information

Application	WB, IP, IF, FCM, ChIP	Dilution	WB: 1:1000; IP: 1:50; IF: 1:400 - 1:1600; FCM: 1:200 - 1:800; CHIP: 1:50
Reactivity	Human, Mouse, Rat		
Source	Rabbit Monoclonal Antibody	MW	60 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
1204. 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.

IF

Experimental Protocol:

Sample Preparation
1. Adherent Cells: Place a clean, sterile coverslip in a culture dish. Once the cells grow to near confluence as a monolayer, remove the coverslip for further use.
2. Suspension Cells: Seed the cells onto a clean, sterile slide coated with poly-L-lysine.
3. Frozen Sections: Allow the slide to thaw at room temperature. Wash it with pure water or PBS for 2 times, 3 minutes each time.
4. Paraffin Sections: Deparaffinization and rehydration. Wash the slide with pure water or PBS for 3 times, 3 minutes each time. Then perform antigen retrieval.

Fixation
1. Fix the cell coverslips/spots or tissue sections at room temperature using a fixative such as 4% paraformaldehyde (4% PFA) for 10-15 minutes.

Experimental Methods

2. Wash the sample with PBS for 3 times, 3 minutes each time.

Permeabilization
1.Add a detergent such as 0.1–0.3% Triton X-100 to the sample and incubate at room temperature for 10–20 minutes.
(Note: This step is only required for intracellular antigens. For antigens expressed on the cell membrane, this step is unnecessary.)
Wash the sample with PBS for 3 times, 3 minutes each time.

Blocking
Add blocking solution and incubate at room temperature for at least 1 hour. (Common blocking solutions include: serum from the same source as the secondary antibody, BSA, or goat serum.)
Note: Ensure the sample remains moist during and after the blocking step to prevent drying, which can lead to high background.

Immunofluorescence Staining (Day 1)
1. Remove the blocking solution and add the diluted primary antibody.
2. Incubate the sample in a humidified chamber at 4°C overnight.

Immunofluorescence Staining (Day 2)
1. Remove the primary antibody and wash with PBST for 3 times, 5 minutes each time.
2. Add the diluted fluorescent secondary antibody and incubate in the dark at 4°C for 1–2 hours.
3. Remove the secondary antibody and wash with PBST for 3 times, 5 minutes each time.
4. Add diluted DAPI and incubate at room temperature in the dark for 5–10 minutes.
5. Wash with PBST for 3 times, 5 minutes each time.

Mounting
1. Mount the sample with an anti-fade mounting medium.
2. Allow the slide to dry at room temperature overnight in the dark.
3. Store the slide in a slide storage box at 4°C, protected from light.

References

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

Application Data

IF

Validated by Capsid Bio



Immunofluorescent analysis of HT1080 cells, serum-starved (overnight) (upper) and Human BMP2-treated (50 ng/ml, 30 min) (lower), using F0347(green, 1:800), Hoechst (blue) and tubulin (Red).

WB

Validated by Capsid Bio



Lane 1: Hep G2
Lane 2: Hep G2 (hBMP2, 50 ng/ml, 30 min)
Lane 3: MEF
Lane 4: MEF (hBMP2, 50 ng/ml, 30 min)