

Anti-Osteopontin Antibody [PSH14-11]

HA723601



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Mouse, Rat
Applications:	IHC-P, IHC-Fr, WB, IF-Cell
Molecular Wt:	Predicted band size: 32 kDa
Clone number:	PSH14-11

Description: Osteopontin (OPN), also known as bone /sialoprotein I (BSP-1 or BNSP), early T-lymphocyte activation (ETA-1), secreted phosphoprotein 1 (SPP1), 2ar and Rickettsia resistance (Ric), is a protein that in humans is encoded by the SPP1 gene (secreted phosphoprotein 1). The murine ortholog is Spp1. Osteopontin is a SIBLING (glycoprotein) that was first identified in 1986 in osteoblasts. The prefix osteo- indicates that the protein is expressed in bone, although it is also expressed in other tissues. The suffix -pontin is derived from "pons," the Latin word for bridge, and signifies osteopontin's role as a linking protein. Osteopontin is an extracellular structural protein and therefore an organic component of bone. The gene has 7 exons, spans 5 kilobases in length and in humans it is located on the long arm of chromosome 4 region 22 (4q1322.1). The protein is composed of ~300 amino acids residues and has ~30 carbohydrate residues attached, including 10 sialic acid residues, which are attached to the protein during post-translational modification in the Golgi apparatus. The protein is rich in acidic residues: 30-36% are either aspartic or glutamic acid.

Immunogen: Recombinant protein within mouse Osteopontin aa 1-294.

Positive control: Mouse knee joint tissue, RAW264.7 treated with 100ng/mL LPS for 4 hours add 1µg/mL BFA for last 3 hours cell lysate, RAW264.7 add 100nM TPA overnight then treated with 100ng/mL LPS for 4 hours add 1µg/mL BFA for last 3 hours cell lysate, RAW264.7 cells treated with 100ng/mL LPS for 4 hours, add 1µg/mL BFA for last 3 hours.

Subcellular location: Secreted.

Database links: SwissProt: P10923 Mouse | P08721 Rat

Recommended Dilutions:

IHC-P	1:9,000
IHC-Fr	1:500
WB	1:5,000
IF-Cell	1:2,000

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

Purity: Protein A affinity purified.

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Orders:0086-571-88062880

Technical:0086-571-89986345

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Images

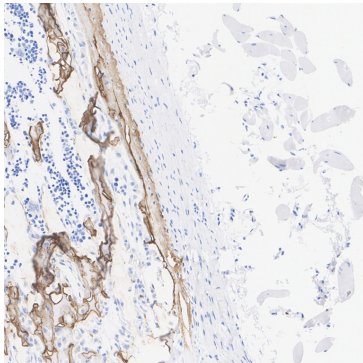


Fig1: Immunohistochemical analysis of paraffin-embedded mouse knee joint tissue with Rabbit anti-Osteopontin antibody (HA723601) at 1/9,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA723601) at 1/9,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Fig2: Application: IHC-Fr

Species: Mouse

Site: kidney

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

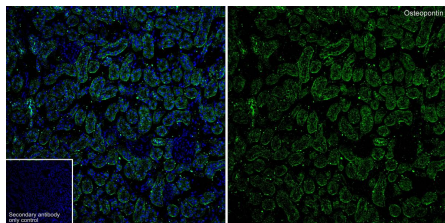


Fig3: Application: IHC-Fr

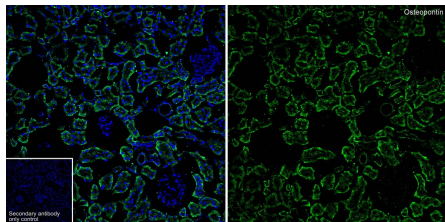
Species: Rat

Site: kidney

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required



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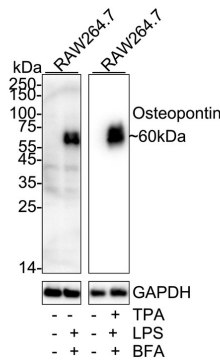
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Fig4: Western blot analysis of Osteopontin on different lysates with Rabbit anti-Osteopontin antibody (HA723601) at 1/5,000 dilution.



Lane 1: RAW264.7 cell lysate

Lane 2: RAW264.7 treated with 100ng/mL LPS for 4 hours, add 1µg/mL BFA for last 3 hours cell lysate

Lane 3: RAW264.7 cell lysate

Lane 4: RAW264.7 add 100nM TPA overnight, then treated with 100ng/mL LPS for 4 hours, add 1µg/mL BFA for last 3 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 32 kDa

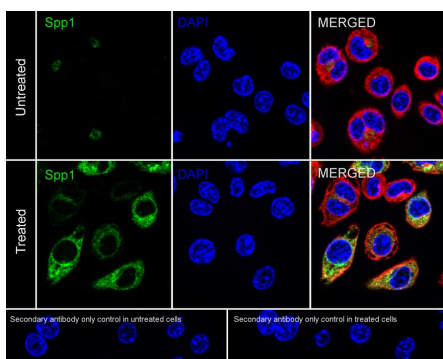
Observed band size: 60 kDa

Exposure time: Lane 1-2: 46 seconds; Lane 3-4: 4 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA723601) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4 °C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig5: Immunocytochemistry analysis of RAW264.7 cells untreated / treated with 100ng/mL LPS for 4 hours, add 1µg/mL BFA for last 3 hours labeling Osteopontin with Rabbit anti-Osteopontin antibody (HA723601) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Osteopontin antibody (HA723601) at 1/2,000 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Han H et al. Macrophage-derived Osteopontin (SPP1) Protects From Nonalcoholic Steatohepatitis. *Gastroenterology*. 2023 Jul
2. Shirakawa K et al. Osteopontin in Cardiovascular Diseases. *Biomolecules*. 2021 Jul

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