Anti-Osteopontin Antibody [PSH09-24] - BSA and Azide free

HA751278

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 35 kDa
Clone number:	PSH09-24

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.

Immunogen: Recombinant protein within human Osteopontin aa 1-314.

Positive control: RAW264.7 treated with 100ng/mL LPS for 4 hours add 1μg/mL BFA for last 3 hours cell lysate, A549 cell lysate, U-87 MG cell lysate, U-87 MG, A549.

Subcellular location: Secreted.

Database links: SwissProt: P10451 Human | P10923 Mouse

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:1,000-1:20,000
FC	1:1,000

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

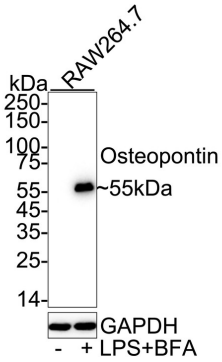

Fig1: Western blot analysis of Osteopontin on different lysates with Rabbit anti-Osteopontin antibody (HA751278) at 1/2,000 dilution.

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

Lysates/proteins at 20 μg/Lane.

Predicted band size: 35 kDa
Observed band size: 55 kDa

Exposure time: 16 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 (HA751278) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

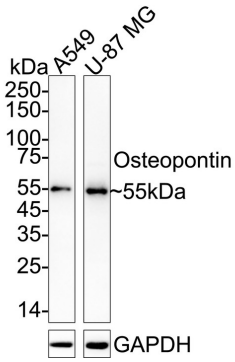

Fig2: Western blot analysis of Osteopontin on different lysates with Rabbit anti-Osteopontin antibody (HA751278) at 1/2,000 dilution.

Lane 1: A549 cell lysate
Lane 2: U-87 MG cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 35 kDa
Observed band size: 55 kDa

Exposure time: 1 minute; ECL: K1802;

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 (HA751278) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

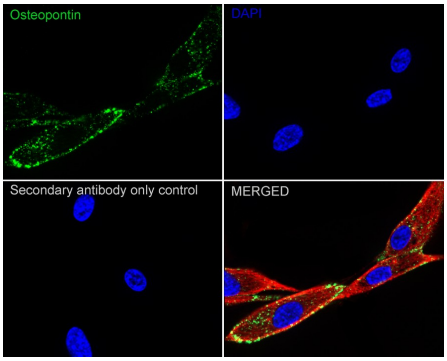

Fig3: Immunocytochemistry analysis of U-87 MG cells labeling Osteopontin with Rabbit anti-Osteopontin antibody (HA751278) at 1/20,000 dilution.

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 (HA751278) at 1/20,000 dilution in 1% BSA in PBST overnight at 4 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

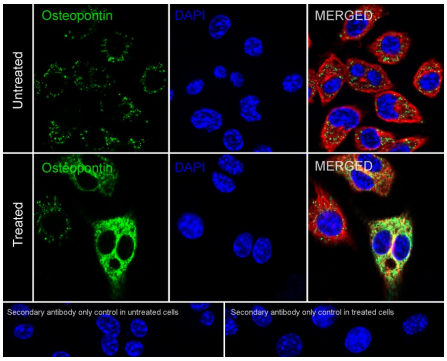

Fig4: Immunocytochemistry analysis of RAW264.7 cells treated with 100ng/mL LPS for 4 hours, add 1μg/mL BFA for last 3 hours labeling Osteopontin with Rabbit anti-Osteopontin antibody (HA751278) at 1/1,000 dilution.

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 (HA751278) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

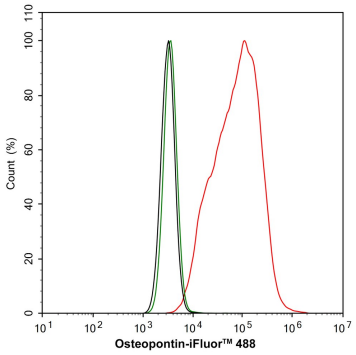

Fig5: Flow cytometric analysis of A549 cells labeling Osteopontin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751278, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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