Anti-Osteoprotegerin Antibody

R1608-4

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	46 kDa

Description: Bone morphogenesis and remodeling involve the formation of bone from osteoblasts and the resorption of bone by osteoclasts. The cytokine osteoprotegerin (OPG), also designated osteoclastogenesis inhibitory factor (OCIF), is known to inhibit osteoclast formation. A secreted glycoprotein, OPG is a member of the TNF receptor family that increases bone density and volume. OPG is thought to inhibit osteoclastogenesis by disrupting the cell-to-cell signaling between osteoblastic stromal cells and osteoclast progenitors. OPG is known to bind to TRAIL, a death domain-containing protein, and to inhibit TRAIL apoptosis in Jurkat cells. OPG also binds to osteoclast differentiation factor (ODF), also known as TRANCE/RANKL, a membrane-bound protein belonging to the TNF ligand family. Both TNFα and TNFβ upregulate OPG expression, while the bone resorbing agent prostaglandin E2 downregulates OPG.

Immunogen: Recombinant protein within Human Osteoprotegerin aa 1-222 / 401.

Positive control: 293 cell lysate, K562 cell lysate, K-562, Neuro-2a, C6, human lung cancer tissue, human breast cancer tissue, human kidney tissue, mouse kidney tissue, mouse brain tissue.

Subcellular location: Secreted.

Database links: SwissProt: O00300 Human | O08712 Mouse | O08727 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:100-1:500
IHC-P	1:50-1:200
FC	1μg/mL

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

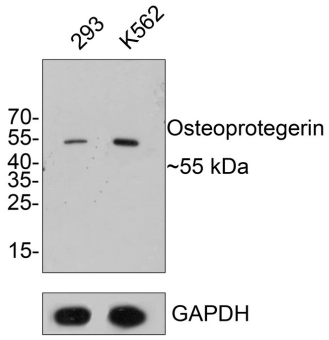

Fig1: Western blot analysis of Osteoprotegerin on different lysates with Rabbit anti-Osteoprotegerin antibody (R1608-4) at 1/1,000 dilution.

Lane 1: 293 cell lysate
Lane 2: K562 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 46 kDa
Observed band size: 55 kDa

Exposure time: 1 minutes;

10% 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 (R1608-4) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

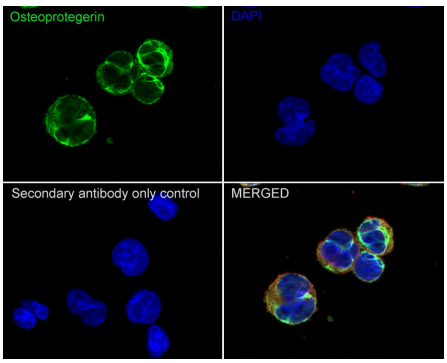

Fig2: Immunocytochemistry analysis of K-562 cells labeling Osteoprotegerin with Rabbit anti-Osteoprotegerin antibody (R1608-4) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Osteoprotegerin antibody (R1608-4) at 1/100 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 (M1305-2, 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.

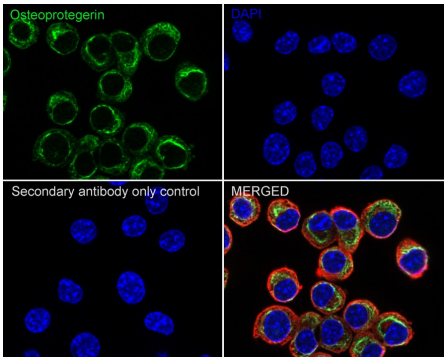

Fig3: Immunocytochemistry analysis of Neuro-2a cells labeling Osteoprotegerin with Rabbit anti-Osteoprotegerin antibody (R1608-4) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Osteoprotegerin antibody (R1608-4) at 1/100 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 (M1305-2, 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.

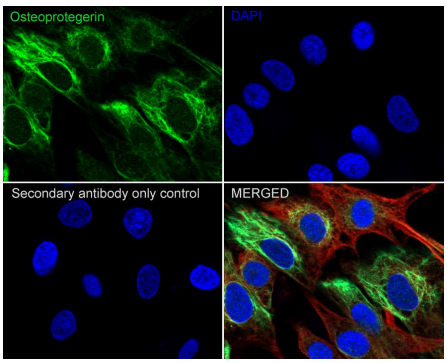

Fig4: Immunocytochemistry analysis of C6 cells labeling Osteoprotegerin with Rabbit anti-Osteoprotegerin antibody (R1608-4) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Osteoprotegerin antibody (R1608-4) at 1/100 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 (M1305-2, 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.

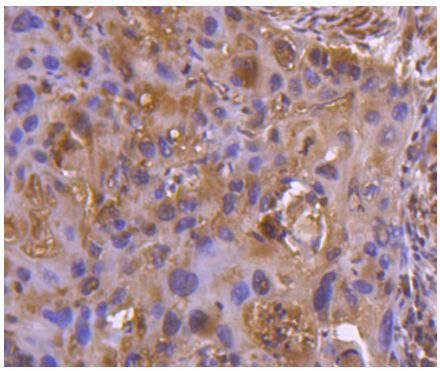

Fig5: Immunohistochemical analysis of paraffin-embedded human lung cancer tissue using anti-Osteoprotegerin antibody. Counter stained with hematoxylin.

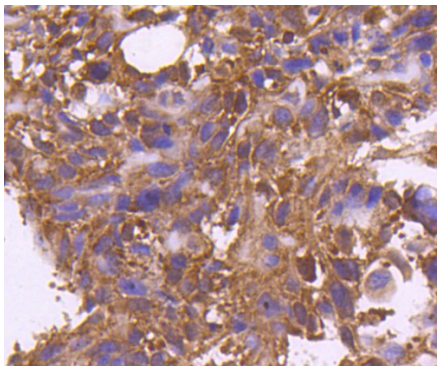

Fig6: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue using anti-Osteoprotegerin antibody. Counter stained with hematoxylin.

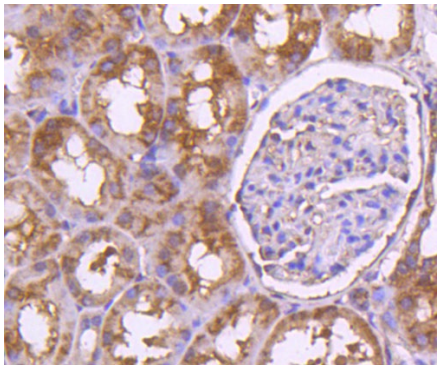

Fig7: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-Osteoprotegerin antibody. Counter stained with hematoxylin.

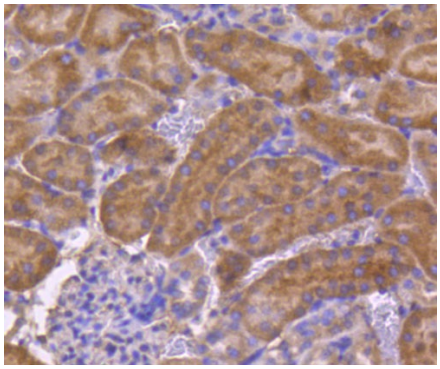

Fig8: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue using anti-Osteoprotegerin antibody. Counter stained with hematoxylin.

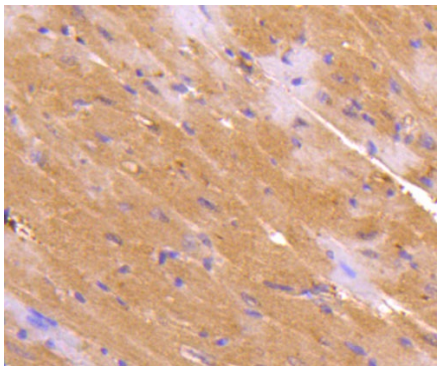

Fig9: Immunohistochemical analysis of paraffin-embedded mouse heart tissue using anti-Osteoprotegerin antibody. Counter stained with hematoxylin.

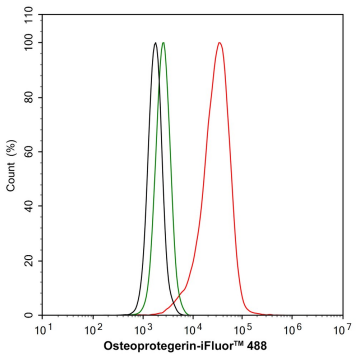

Fig10: Flow cytometric analysis of K-562 cells labeling Osteoprotegerin.

Cells were fixed and permeabilized. Then stained with the primary antibody (R1608-4, 1μg/mL) (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).

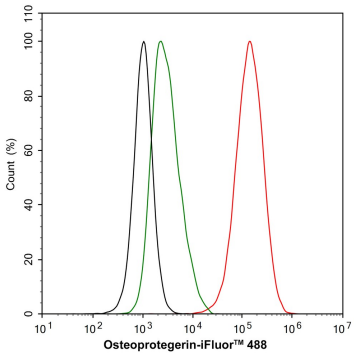

Fig11: Flow cytometric analysis of C6 cells labeling Osteoprotegerin.

Cells were fixed and permeabilized. Then stained with the primary antibody (R1608-4, 1μg/mL) (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).

Background References

1. Bao Y et al. Topical Treatment with Xiaozheng Zhitong Paste (XZP) Alleviates Bone Destruction and Bone Cancer Pain in a Rat Model of Prostate Cancer-Induced Bone Pain by Modulating the RANKL/RANK/OPG Signaling. Evid Based Complement Alternat Med 2015:215892 (2015).
2. Peng X et al. Differential expression of the RANKL/RANK/OPG system is associated with bone metastasis in human non-small cell lung cancer. PLoS One 8:e58361 (2013).