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Datasheet for 100-401-404

Osteopontin Antibody

Overview

Description:	Anti-Osteopontin (RABBIT) Antibody - 100-401-404
Item No.:	100-401-404
Size:	200 µL
Applications:	ELISA, IHC, WB, Other
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:

Anti-Osteopontin Antibody recognizes Osteopontin (OPN) which is an arginine-glycine-aspartic acid (RGD)-containing glycoprotein that interacts with integrins and CD44 as major receptors. OPN is multifunctional, with activities in cell migration, cell survival, inhibition of calcification, regulation of immune cell function, and control of tumor cell phenotype. The gene encoding OPN is called spp1. Targeting this gene has revealed that while OPN is not necessary for normal embryonic development, fertility, and health under pathogen-free conditions, loss of the protein has significant consequences in several models of injury/disease as diverse as renal injury, viral, and bacterial infection, bone remodeling, and tumor growth. The fact that no other proteins seem to share a redundant activity with OPN under these conditions suggests that OPN has a unique functional role during tissue injury and stress. Interestingly, several members of the matrix metalloproteinase (MMP) family are also induced during injury/disease processes in patterns overlapping that of OPN. OPN has recently been shown to be a novel substrate for two MMPs, MMP-3 (stromelysin-1) and MMP-7 (matrilysin). There are three cleavage sites for MMP-3 in human OPN, two of which are also cleaved by MMP-7 (see cleavage diagram). Biological assays demonstrate that the MMP-cleaved OPN has increased activity in promoting both cell adhesion and migration compared with full-length OPN. In addition, inhibitory reagents were used to show that the same receptors that interact with OPN also mediate interaction of MMP-cleaved OPN with tumor cells. It is suggested that active forms of OPN at sites of tissue injury may be regulated by the activity of proteases including MMPs and that the differences in activity of modified OPN may be explained by differences in binding affinity of integrins or distinct downstream signaling events. Osteopontin can be responsible for diseases such as lung and prostate cancers, nephrolithiasis, hepatocellular carcinoma, osteoporosis and arteriosclerosis. Anti-Osteopontin is useful for researchers interested in Stem Cell and Extracellular Matrix Antibodies.

Synonyms:	rabbit anti-Osteopontin antibody, Osteopontin Antibody, Bone sialoprotein 1, Secreted phosphoprotein 1, SPP-1, Urinary stone protein, Nephropontin, Uropontin, SPP1, BNSP, OPN, PSEC0156
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

Target Details

Gene Name:	SPP1
Reactivity:	Human
Immunogen Type:	Conjugated Peptide
Immunogen:	This whole rabbit serum was prepared by repeated immunizations with a synthetic peptide, from the human osteopontin protein, conjugated to KLH using maleimide.
Purity/Specificity:	Osteopontin is directed against human osteopontin. The antibody recognizes the full-length osteopontin protein (which runs at 66 kDa on westerns), as well as the C-terminal fragments of both thrombin and MMP-cleaved OPN. The 32 kDa MMP-cleaved C-fragment is recognized, but not the 40 kDa N-terminal fragment. Reactivity is reported to occur with osteopontin from swine, dog, mouse and rat.
Relevant Links:	• UniProtKB - P10451 • NCBI - AAA59974.1 • GeneID - 6696

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	Other (Based on references)
Application Note:	Anti-Osteopontin has been tested for western blotting, immunohistochemistry (formalin-fixed paraffin-embedded sections) and ELISA. The antibody exclusively recognizes C-terminal fragments of both thrombin and MMP-cleaved OPN. The antibody recognizes the full-length osteopontin protein (which runs at 66 kDa on westerns), and 32 kDa for the MMP-cleaved C-fragment, but the 40 kDa N-terminal fragment is not recognized. A 1:1000 dilution will detect strongly approximately 250 ng of OPN protein on a blot. A 1:100-1:300 dilution used for IHC on human breast tumor tissues. No pretreatment is required for IHC when formalin-fixed paraffin-embedded tissue is stained. Specific conditions for reactivity and signal detection should be optimized by the end user.

Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5,000 - 1:20,000
IHC:	1:100 - 1:300
IP:	1:100
WB:	1:500 - 1:2,000

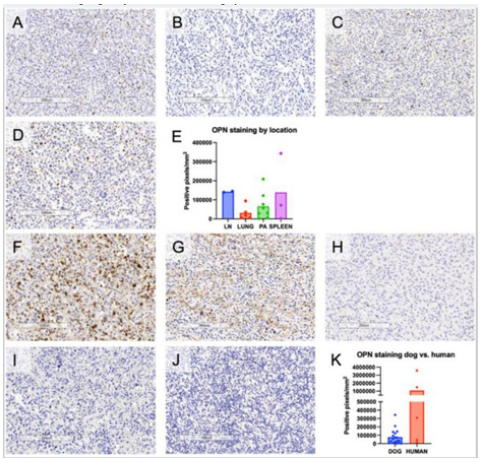
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	95 mg/mL by Refractometry
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

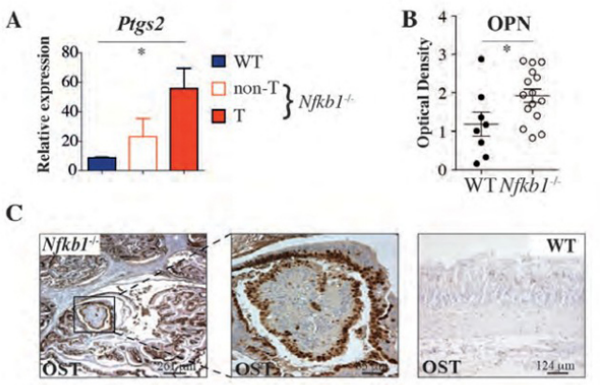
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

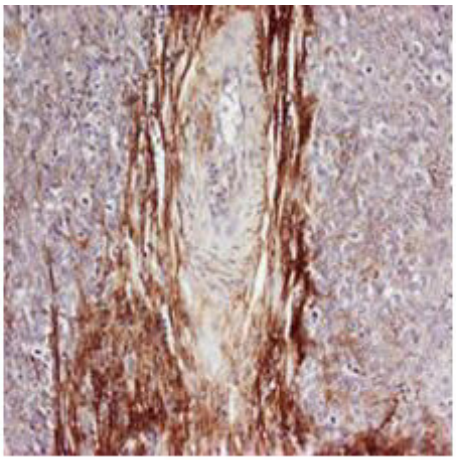
Images



Immunohistochemistry
Immunostaining of canine and human histiocytic sarcoma (HS) for osteopontin. Formalin-fixed paraffin-embedded tissues from 18 canine HS and five human HS were stained for osteopontin expression. Slides scanned using an Aperio AT2 automated slide scanner (Leica Biosystems) were visualized using ImageScope software (Leica Biosystems) and an algorithm for quantifying positive pixels was applied to non-necrotic areas of the entirety of the outlined tumor area. Photomicrographs of representative canine HS with primary tissue of origin of A lymph node (LN), B lung, C peri-articular (PA) tissues and D spleen. E Quantification of osteopontin staining grouped by anatomic location in 18 canine HS samples. Each dot represents an individual tumor, and the bar represents the anatomic mean. Photomicrographs of human tumors from five patients diagnosed with HS in F the brain, G subcutaneous tissue of the buttock, H subcutaneous tissue of the foot, I spleen and J tonsil. K Quantification of osteopontin staining grouped by species. Each dot represents an individual tumor, and the bar represents the species mean. Scale bars measure 200 μm.
Fig 2. PMID: 39751954.



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Immunohistochemistry
Rabbit anti-Osteopontin was used at a 1:100-1:300 dilution to detect osteopontin by immunohistochemistry. Osteopontin is a normal component of elastic fibers in the breast (shown heavily stained in this section of human breast tumor cells). There is also weak staining of the extracellular matrix. Osteopontin is not expressed in breast tumor cells, and there is no staining of the breast cells in this section. No antigen retrieval is required.

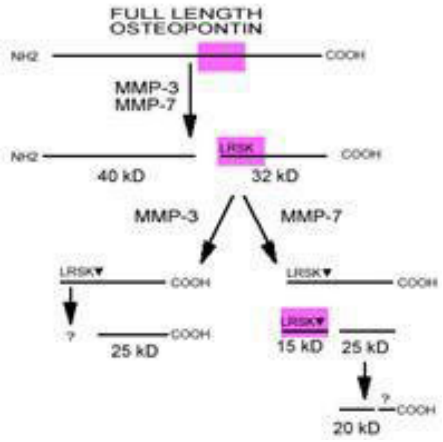
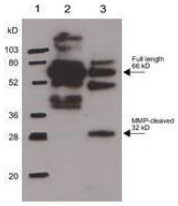


Diagram
OPN is cleaved by MMP to yield 2 fragments, which migrate at 40 kDa (N terminal) and 32 kDa (C terminal). The C terminal fragment can undergo further cleavage by both of these MMPs. The epitope that is recognized by Rabbit-anti-Osteopontin is shown in violet. This antibody detects the full length OPN and the 32 kDa fragment. It does not recognize the 40 kDa fragment.



Western Blot
Western Blot of Rabbit Anti-Osteopontin Antibody. Lane 1: Molecular Weight Marker. Lane 2: Human Osteopontin [250ng]. Lane 3: MMP-cleaved Osteopontin. Primary Antibody: Anti-Osteopontin at 1:1000 ovenight at 2-8°C. Secondary Antibody: Goat Anti-Rabbit IgG HRP (p/n 611-103-122) at 1:10,000. Observed MW: full length 66kDa, MMP-cleaved 32kDa.

References

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- Cherepanova et al. Activation of the pluripotency factor OCT4 in smooth muscle cells is atheroprotective. *Nature Medicine* (2016)
- Kim S, Shin T. Immunohistochemical study of osteopontin in boar testis. *J Vet Sci.* (2007)

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