

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Osteopontin/OPN in direct ELISAs and Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Human milk-derived Osteopontin/OPN
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

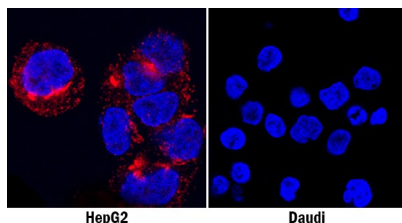
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Dual RNAscope ISH-IHC Compatible	5-15 µg/mL	Immersion fixed paraffin-embedded sections of human kidney
Immunocytochemistry	1-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Neutralization	Measured by its ability to neutralize Osteopontin/OPN-mediated adhesion of the HEK293 human embryonic kidney cell line. Hu, D.D. et al. (1995) J. Biol. Chem. 270 :26232. The Neutralization Dose (ND ₅₀) is typically 0.300 - 3.60 µg/mL in the presence of 1 µg/mL Recombinant Human Osteopontin/OPN.	
ELISA	This antibody functions as an ELISA detection antibody when paired with Mouse Anti-Human Osteopontin/OPN Monoclonal Antibody (Catalog # MAB14332R). <i>This product is intended for assay development on various assay platforms requiring antibody pairs. We recommend the Human Osteopontin (OPN) DuoSet ELISA Kit (Catalog # DY1433) for convenient development of a sandwich ELISA or the Human Osteopontin (OPN) Quantikine ELISA Kit (Catalog # DOST00) for a complete optimized ELISA.</i>	

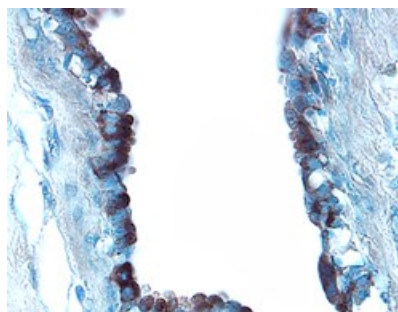
DATA

Immunocytochemistry



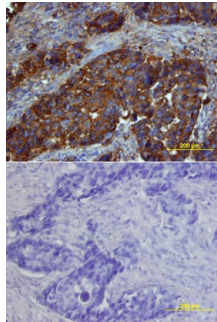
Osteopontin/OPN in HepG2 Human Cell Line.
Osteopontin/OPN was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line (positive staining; left panel) and Daudi human Burkitt's lymphoma cell line (negative staining; right panel) using Goat Anti-Human Osteopontin/OPN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1433) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # [NL001](#)) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry



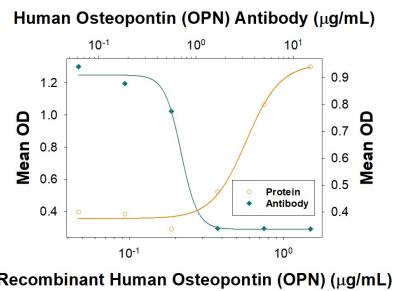
Osteopontin/OPN in Human Breast Cancer Tissue.
Osteopontin/OPN was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using 8 µg/mL Goat Anti-Human Osteopontin/OPN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1433) overnight at 4 °C. Tissue was stained with the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # [CTS008](#)) and counterstained with hematoxylin (blue). Specific labeling was localized to the surface of epithelial cells in the intralobular duct. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

Immunohistochemistry



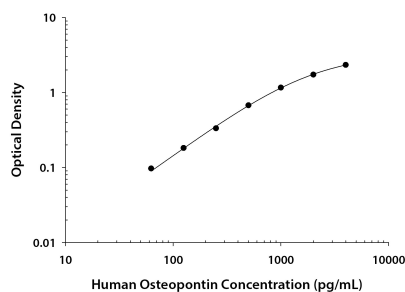
Osteopontin/OPN in Human Breast Cancer Tissue. Osteopontin/OPN was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using Goat Anti-Human Osteopontin/OPN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1433) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

Neutralization



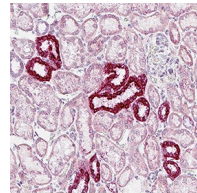
Cell Adhesion Mediated by Osteopontin/OPN and Neutralization by Human Osteopontin/OPN Antibody. Recombinant Human Osteopontin/OPN (Catalog # Catalog # 1433-OP), immobilized onto a microplate, supports the adhesion of the HEK293 human embryonic kidney cell line in a dose-dependent manner (orange line). Adhesion elicited by Recombinant Human Osteopontin/OPN (1 µg/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human Osteopontin/OPN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1433). The ND₅₀ is typically 0.300 - 3.60 µg/mL.

ELISA

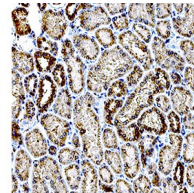


Human Osteopontin/OPN ELISA Standard Curve. Recombinant Human Osteopontin/OPN protein was serially diluted 2-fold and captured by Mouse Anti-Human Osteopontin/OPN Monoclonal Antibody (Catalog # Catalog # MAB14332R) coated on a Clear Polystyrene Microplate (Catalog # Catalog # DY990). Goat Anti-Human Osteopontin/OPN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1433) was biotinylated and incubated with the protein captured on the plate. Detection of the standard curve was achieved by incubating Streptavidin-HRP (Catalog # Catalog # DY998) followed by Substrate Solution (Catalog # Catalog # DY999) and stopping the enzymatic reaction with Stop Solution (Catalog # Catalog # DY994).

In-situ Hybridization



In Situ Hybridization (ISH)



Immunohistochemistry (IHC)

Detection of Osteopontin/OPN in Human Kidney. Formalin-fixed paraffin-embedded tissue sections of human kidney were probed for OPN mRNA (ACD RNAScope Probe, catalog #420101; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using goat anti-human OPN polyclonal antibody (R&D Systems catalog # Catalog # AF1433) at 1µg/mL with overnight incubation at 4 degrees Celsius followed by incubation with anti-goat IgG VisUCyte HRP Polymer Antibody (Catalog # Catalog # VC004) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in tubules.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Osteopontin (OPN, previously also referred to as transformation-associated secreted phosphoprotein, bone sialoprotein I, 2ar, 2B7, early T lymphocyte activation 1 protein, minopotin, calcium oxalate crystal growth inhibitor protein), is a secreted, highly acidic, calcium-binding, RGD-containing, phosphorylated glycoprotein originally isolated from bone matrix (1). Subsequently, OPN has been found in kidney, placenta, blood vessels and various tumor tissues. Many cell types (including macrophages, osteoclasts, activated T cells, fibroblasts, epithelial cells, vascular smooth muscle cells, and natural killer cells) can express OPN in response to activation by cytokines, growth factors or inflammatory mediators. Elevated expression of OPN has also been associated with numerous pathobiological conditions such as atherosclerotic plaques, renal tubulointerstitial fibrosis, granuloma formations in tuberculosis and silicosis, neointimal formation associated with balloon catheterization, metastasizing tumors, and cerebral ischemia. Human OPN cDNA encodes a 314 amino acid (aa) residue precursor protein with a 16 aa residue predicted signal peptide that is cleaved to yield a 298 aa residue mature protein with an integrin binding sequence (RGD), and N- and O-glycosylation sites. By alternative splicing, at least three human OPN isoforms exist. OPN has been shown to bind to different cell types through RGD-mediated interaction with the integrins $\alpha\beta_1$, $\alpha\beta_3$, $\alpha\beta_5$, and non-RGD-mediated interaction with CD44 and the integrins $\alpha_8\beta_1$ or $\alpha_9\beta_1$. OPN exists both as a component of extracellular matrix and as a soluble molecule. Functionally, OPN is chemotactic for macrophages, smooth muscle cells, endothelial cells and glial cells. OPN has also been shown to inhibit nitric oxide production and cytotoxicity by activated macrophages. Human, mouse, rat, pig, and bovine OPN share from approximately 40-80% amino acid sequence identity. Osteopontin is a substrate for proteolytic cleavage by thrombin, enterokinase, MMP-3, and MMP-7. The functions of OPN in a variety of cell types were shown to be modified as a result of proteolytic cleavage (2, 3).

References:

1. Ann. N.Y. Acad. Sci. (1995) **760**, Apr. 21.
2. Senger, D.R. *et al.* (1996) Biochim. Biophys. Acta. **1314**:13.
3. Agnihotri, R. *et al.* (2001) J. Biol. Chem. **276**:28261.