Human Osteopontin/OPN Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1433

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Osteopontin/OPN in direct ELISAs and Western blots. In direct ELISAs, less than 10% cross-reactivity with recombinant rat Osteopontin, bovine Osteopontin and recombinant mouse OPN is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Human milk-derived Osteopontin/OPN
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
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 as a 0.2 µm filtered solution in PBS.

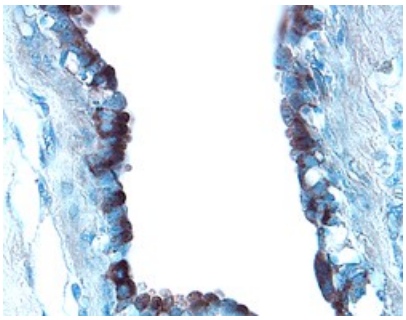
APPLICATIONS

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
Western Blot	0.1 µg/mL	Recombinant Human Osteopontin/OPN (Catalog # 1433-OP)
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 2-6 µg/mL in the presence of 1 µg/mL Recombinant Human Osteopontin/OPN.	

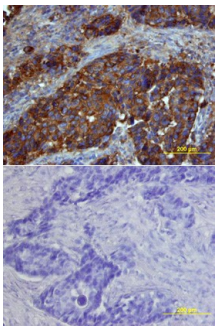
DATA

Immunohistochemistry



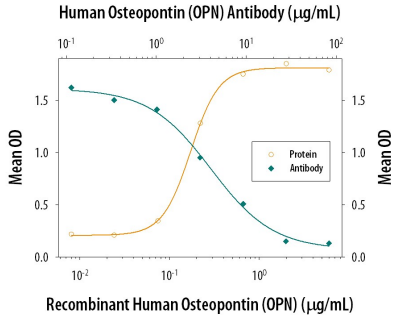
Osteopontin/OPN in Human Breast Cancer Tissue. Osteopontin/OPN was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using 8 µg/mL Goat Anti-Human Osteopontin/OPN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1433) overnight at 4 °C. Tissue was stained 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 # 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 ND_{50} is typically 2-6 µg/mL.

bio-techne
bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956

Human Osteopontin/OPN Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1433

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 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 $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, 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.

bio-techne
bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956