Mouse Osteopontin/OPN Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF808

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse Osteopontin (OPN) in ELISAs and Western blots. In sandwich ELISAs, less than 3% cross-reactivity with recombinant human OPN is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse Osteopontin/OPN (R&D Systems, Catalog # 441-OP) Leu17-Asn294 (Glu99Gly) Accession # Q547B5
Endotoxin Level	<0.30 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 either lyophilized or as a 0.2 µm filtered solution in PBS.

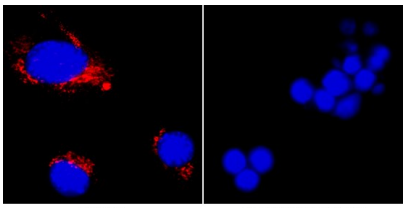
APPLICATIONS

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
Western Blot	0.1 µg/mL	Recombinant Mouse Osteopontin/OPN (Catalog # 441-OP)
Immunocytochemistry	5-25 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Mouse Osteopontin/OPN Sandwich Immunoassay		**Reagent**
ELISA Capture	0.2-0.8 µg/mL	Mouse Osteopontin/OPN Antibody (Catalog # AF808)
ELISA Detection	0.1-0.4 µg/mL	Mouse Osteopontin/OPN Biotinylated Antibody (Catalog # BAF808)
Standard		Recombinant Mouse Osteopontin/OPN (Catalog # 441-OP)
Neutralization	Measured by its ability to neutralize Osteopontin/OPN-mediated adhesion of the HEK293 human embryonic kidney cell line. The Neutralization Dose (ND_{50}) is typically 1-3 µg/mL in the presence of 2 µg/mL Recombinant Mouse Osteopontin/OPN.	

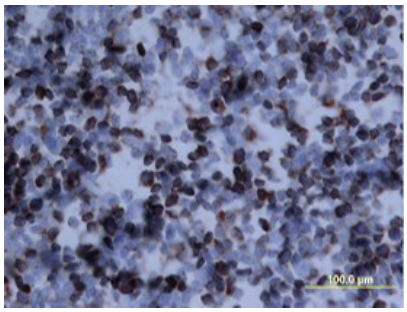
DATA

Immunocytochemistry



Osteopontin/OPN in C2C12 Mouse Cell Line and Mouse Splenocytes. Osteopontin/OPN was detected in immersion fixed C2C12 mouse myoblast cell line (left panel, positive stain) and mouse splenocytes (right panel, negative stain) using Goat Anti-Mouse Osteopontin/OPN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF808) at 5 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 and secreted molecule. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



Osteopontin/OPN in Mouse Thymus. Osteopontin/OPN was detected in perfusion fixed frozen sections of mouse thymus using Mouse Osteopontin/OPN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF808) 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). View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

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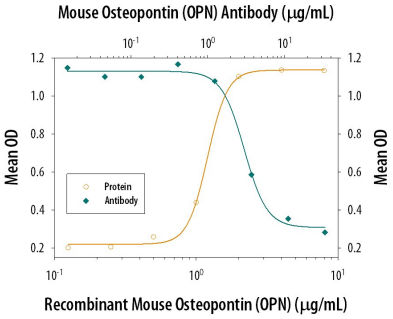
Neutralization



Cell Adhesion Mediated by Osteopontin/OPN and Neutralization by Mouse Osteopontin/OPN Antibody. Recombinant Mouse Osteopontin/OPN (Catalog # 441-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 Mouse Osteopontin/OPN (2 µg/mL) is neutralized (green line) by increasing concentrations of Mouse Osteopontin/OPN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF808). The ND_{50} is typically 1-3 µg/mL.

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. 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. Mouse OPN cDNA encodes a 294 amino acid (aa) residue precursor protein with a 16 aa residue predicted signal peptide that is cleaved to yield a 278 aa residue mature protein with an integrin binding sequence (RGD), and N- and O-glycosylation sites. 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 $\alpha_9\beta_1$. 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., vol. 760, 1995, 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.

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