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Product Information

Anti-Osteopontin
produced in goat, affinity isolated antibody

Catalog Number **O7635**

Synonym: Anti-OPN

Product Description

Anti-Osteopontin is produced in goat using a purified recombinant mouse osteopontin, expressed in NSO cells as immunogen. Affinity isolated antibody is obtained from goat anti-osteopontin antiserum by immuno-specific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the osteopontin.

Anti-Osteopontin recognizes recombinant mouse OPN by various immunochemical techniques including immunoblotting, capture ELISA, immunohistochemistry, and neutralization.

Mouse osteopontin,[1] a 284 amino acid residue protein, has a calculated molecular mass of 31.5 kDa. As a result of glycosylation, recombinant mouse OPN migrates as a doublet (65 kDa) and another band (30 kDa) in SDS-PAGE under reducing conditions. At the amino acid level, human, mouse, rat, pig, and bovine OPN are approximately 40% identical.

Osteopontin (OPN), also known as transformation-associated secreted phosphoprotein, bone sialoprotein I, 2ar, 2B7, early T lymphocyte activation protein-1 (Eta-1), minopotin, and calcium oxalate crystal growth inhibitor, is a secreted, highly acidic, calcium binding, phosphorylated glycoprotein. Native mouse OPN cDNA encodes a 294 amino acid residue precursor protein with a 16 amino acid residue predicted signal peptide that is cleaved to yield a 278 amino acid residue mature protein with an intergrin binding sequence (RGD), a thrombin cleavage site, and N- and O-glycosylation sites. OPN binds various cell types through RGD-mediated interaction with the integrins $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, and non-RGF-mediated interaction with CD44 and intergrins ($\alpha_8\beta_1$ or $\alpha_9\beta_1$).[2]

Osteopontin, originally isolated from bone matrix, is also found in kidney, placenta, blood vessels, and various tumor tissues. Many cell types (macrophages, osteoclasts, activated T-cells,[3] fibroblasts, epithelial cells, vascular smooth muscle cells, and natural killer cells) express osteopontin in response to activation by cytokines, growth factors, or inflammatory mediators. In activated macrophages, OPN inhibits nitric oxide production and cytotoxicity. Increased expression of OPN is associated with numerous pathobiological conditions such as atheroschlerotic plaques, renal tubulointerstitial fibrosis, granuloma formations in tuberculosis and silicosis,[4] neointimal formation associated with balloon catheterization, metastasizing tumors, and cerebral ischemia. OPN is chemotactic for macrophages, smooth muscle cells, endothelial cells, and glial cells.

The murine Eta-1 (identical to osteopontin) gene maps to chromosome 5.[5]

Reagent

Supplied as ~100 µg of antiserum lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 5% trehalose.

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile phosphate buffered saline (PBS) to produce a 0.1 mg/ml stock solution of antibody.

Storage/Stability

Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Do not store in a frost-free freezer.

Product Profile

Neutralization: this antibody has the ability to neutralize the bioactivity of recombinant mouse osteopontin. Immobilized recombinant mouse osteopontin (2 μg/ml) is incubated with various concentrations of the antibody (0.01 μg/ml to 40 μg/ml) for 1 hour at 37 °C in a 96 well plate.[6] Following this preincubation period, 293 cells ($1x10^6$ cells/ml) (100 μl /well) is added. The total mixture is incubated at 37 °C for 45 minutes in a humidified CO_2 incubator. At the end of the incubation, non-adherent cells are washed off. The cells attached to the wells are detected by measuring endogenous cellular lysosomal acid phosphatase activity.

The Neutralization $Dose_{50}$ (ND_{50}) for Anti-Osteopontin is 1-3 μg/ml in the presence of 2 μg/ml of recombinant mouse osteopontin using 293 cells.

The ND_{50} is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when the cytokine is present at a concentration just high enough to elicit a maximum response.

The exact concentration of antibody required to neutralize mouse osteopontin activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

Capture ELISA: using plates coated with 100 μL/well of the capture antibody at 0.2-0.8 μg/mL in combination with 100 μl/well of the detection antibody, an ELISA for samples volumes of 100 μL can be obtained. Titrate each preparation of the recombinant protein for standard preparation to obtain the most suitable dose range. A two-fold dilution series starting at 1 ng/mL is recommended. The antibody shows <3% cross-reactivity with recombinant human osteopontin.

Immunoblotting: a working antibody concentration of 0.1-0.2 μg/mL is recommended. The detection limit for recombinant mouse osteopontin is ~1 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry: a working antibody concentration of 5-15 μg/mL is recommended using cells and tissues.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

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