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Product Information

Anti-Osteopontin
produced in goat, affinity isolated antibody

Catalog Number **O3389**

Product Description

Anti-Osteopontin is produced in goat using as immunogen purified recombinant human osteopontin (OPN). The antibody is purified by human osteopontin affinity chromatography.

Anti-Osteopontin recognizes recombinant human osteopontin by various immunochemical techniques including immunoblotting, immunohistochemistry, and neutralization. The anibody shows less than 5% cross-reactivity with recombinant mouse osteopontin.

Osteopontin (OPN), also known as secreted phosphoprotein 1 (Spp1), bone sialoprotein-1, and early T lymphocyte activation protein-1 (ETA-1), is a secreted acidic phosphorylated glycoprotein. Osteopontin has important functions in bone metabolism and inflammatory processes.[1] 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 interactions with CD44 variants and integrins ($\alpha_8\beta_1$ or $\alpha_9\beta_1$).[2]

Osteopontin (OPN), 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.

Reagent

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of 0.2 μm filtered solution of 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 of Bioactivity

To measure the ability of this antibody to neutralize the bioactivity of human osteopontin, immobilized recombinant human osteopontin (1 μg/mL) is incubated with various concentrations of the antibody (0.1-100 μg/mL) for 1 hour at 37 °C in a 96 well plate. Following this preincubation period, 100 μl of 293 cells ($1x10^6$ cells/mL) is added to each well. 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 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 human osteopontin activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

Immunoblotting: a working antibody concentration of 0.1-0.2 μg/ml is recommended. The detection limit for recombinant human osteopontin is ~2 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry: a working antibody concentration of 5-15 μg/ml is recommended using fixed cells. Cells are fixed with 4% paraformaldehyde in phosphate buffered saline at room temperature for 20 minutes, then blocked with 0.1% Triton® X-100, 1% bovine albumin serum, 10% normal donkey serum in PBS at room termperature for 45 minutes. After blocking, cells are incubated with the diluted primary antibody overnight at 4 °C followed by an appropriate secondary antibody at room temperature for an hour. Between each step, cells are washed with 0.1% BSA in PBS.

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

Endotoxin: < 0.1 EU (endotoxin units)/μg antibody as determined by the LAL (Limulus amebocyte lysate) method.

References

1. Denhardt, D.T., et al., *J. Clin. Invest*., 107, 1055-1061 (2001).
2. Ashkar, S., et al., *Science*, **287**, 860 (2000).
3. Weber, G.F., and Cantor, H., *Cytokine Growth Factor Rev*., **7**, 241 (1996).
4. Nau, G.J., et al., *Proc. Natl. Acad. Sci. USA*, **94**, 6414 (1997).

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