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Product Information

Anti-Osteoprotegerin
produced in goat, affinity isolated antibody

Catalog Number **O1139**

Product Description

Anti-Osteoprotegerin (OPG) is produced in goat using as immunogen purified recombinant mouse osteoprotegerin expressed in mouse NSO cells. Affinity isolated antibody is obtained from goat anti-osteoprotegerin antiserum by immunospecific purification, which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Osteoprotegerin will neutralize the biological activity of recombinant mouse OPG. The antibody may also be used for immunoblotting and ELISA. By immunoblotting (non-reducing), the antibody shows approximately 25% cross-reactivity with recombinant human OPG.

Osteoprotegerin, a member of the tumor necrosis factor receptor (TNFR) superfamily, is a soluble secreted protein that possesses no apparent cell-associated motifs. It is also referred to as osteoclastogenesis inhibitory factor (OCIF) and TNFRSF11B (TNF receptor superfamily, member 11B). Like many other TNFRs, the amino-terminal portion contains four cysteine-rich repeats and the carboxy-terminal portion contains two death domain (DD) homologous motifs. Reduced mouse osteoprotegerin/Fc has a calculated molecular mass of approximately 70.9 kDa. As a result of glycosylation, the recombinant protein migrates as a 100 kDa band in SDS-PAGE under reducing conditions. Human and mouse OPG share ~84% and ~94% homology with rat OPG.

OPG was originally isolated by sequence homology as a TNF receptor family protein in fetal rat intestine and OCIF (initially believed to be a unique cytokine) was isolated from human embryonic fibroblasts.[1,2] The only two ligands currently known for OPG are RANKL (OPGL, ODF, TRANCE) and TRAIL (TNF-related apoptosis-inducing ligand; APO2-L, /apoptosis-2 ligand). TRAIL, which is also a membrane bound signaling receptor, is broadly expressed in a variety of tissues, but not in liver. TRAIL induces apoptosis independent of Fas. TRAIL can apparently neutralize the action of RANKL (OPGL) on OPG by competitive displacement.[3]

The roles of OPG and RANKL in osteoclastogenesis,[4] apoptosis,[5,6] and the functioning immune system[7] are under active investigation. Apparently the balance between OPG and RANKL is a key determinant in whether new bone tissue is formed or existing bone tissue is lost. In recent studies, daily injections of OPG into normal rats remarkably increased bone mineral density and bone volume and decreased the number of osteoclasts. Glucocorticoids, which can cause bone loss, inhibit gene expression for OPG and stimulate production of RANKL.[8] In contrast, estrogen, which helps prevent osteoporosis in menopausal women, stimulates expression of the OPG gene.[9] Injections of OPG also prevented bone and cartilage destruction in mice treated to develop arthritis, but did not prevent inflammation.[10] OPG or a variant of OPG engineered to be a more efficacious drug has the potential to stop bone loss in osteoporosis and T cell disorders.

Mouse OPG transcripts are expressed in liver, lung, heart, and kidney tissue. OPG mRNA is expressed at high levels in stomach, intestines, skin, and calvaria. In humans, high levels are detected in the lung, heart, kidney, and placenta.

Reagent

Lyophilized from 0.2 μm-filtered solution in phosphate buffered saline containing carbohydrates.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the 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 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 prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing. Do not store in a frost-free freezer.

Product Profile

Neutralization of Bioactivity: To measure the ability of the antibody to neutralize the bioactivity of mouse OPG, soluble recombinant mouse: OPG/Fc is incubated with various concentrations of the antibody in a medium containing actinomycin D for 1 hour at 37 °C in 96 well plates. Cross-linked recombinant human TRAIL is then added and incubated for an additional 30 minutes. Following preincubation, the mixture is added to confluent cultures of L929 cells in 96 well plates. The assay mixture (total volume of 150 µl/well) over a monolayer of L929 cells containing antibody (concentrations of 0.01-100 µg/ml), soluble recombinant mouse OPG/Fc (0.1 µg/ml), actinomycin D (1 µg/ml), and cross-linked human TRAIL (20 ng/ml) is incubated at 37 °C for 24 hours in a humidified CO_2 incubator. At the end of the incubation period, the medium is removed and the cells are fixed with 5% formaldehyde and then stained with crystal violet. The stain is subsequently dissolved in 100 µL of 33% acetic acid and the optical density is read at 540 nm.

The Neutralization $Dose_{50}$ (ND_{50}) is 0.5-2 µg/ml in the presence of 0.1 µg/ml of soluble recombinant mouse OPG/Fc, using the TRAIL-sensitive mouse L929 cytolytic assay.

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 recombinant mouse OPG/Fc activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

ELISA: a working antibody concentration of 0.5-1.0 µg/ml is recommended. The detection limit for mouse osteoprotegerin is ~0.5 ng/well.

Immunoblotting: a working antibody concentration of 0.1-0.2 µg/ml is determined using mouse OPG at ~2 ng/lane and 5 ng/lane under non-reducing and reducing conditions, respectively.

Note: In order to obtain the best results in various techniques and preparations, determination of optimal working dilutions by titration test is recommended.

Endotoxin level is <1 EU per 1 µg of the antibody as determined by the LAL (*Limulus* amebocyte lysate) method.

References

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