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Product Information

Anti-Osteoprotegerin
produced in goat, affinity isolated antibody

Catalog Number **O7262**

Synonym: Anti-OPG

Product Description
Anti-Osteoprotegerin is produced in goat using purified recombinant human osteoprotegerin (rhOPG), expressed in the insect cell line *Sf*21 as immunogen. The antibody is purified using human OPG affinity chromatography.

Anti-Osteoprotegerin will neutralize the biological activity of recombinant human OPG. The antibody may also be used for immunoblotting. By immunoblotting (non-reducing), the antibody shows 50% cross-reactivity with recombinant mouse OPG.

Osteoprotegrin, also known as osteoclastogenesis inhibitory factor (OCIF), is a soluble secreted member of the TNFR superfamily that lacks cell attachment motifs. 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. Natural OPG exists predominantly as a disulfide-linked dimer. The only two ligands currently known for OPG are RANKL (OPGL, ODF, TRANCE) and TRAIL (APO2-L, TNF-related apoptosis-inducing ligand/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.

The roles of OPG and RANKL in osteoclastogenesis, apoptosis, and the functioning immune system 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. In contrast, estrogen, which helps prevent osteoporesis in menopausal women, stimulates expression of the OPG gene. Injections of OPG also prevented bone and cartilage destruction in mice treated to develop arthritis, but did not prevent inflammation. 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. OPG and RANKL are undergoing clinical trials for use in osteoporosis and related disorders.

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 PBS to produce a 0.1 mg/ml stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Storage/Stability
Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2-8 °C for at least one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing.

Product Profile

Neutralization of Bioactivity: To measure the ability of the antibody to neutralize the bioactivity of human OPG, soluble recombinant human OPG was incubated with various concentrations of the antibody in medium containing actinomycin D for 60 minutes at 37 °C. Cross-linked recombinant human TRAIL was then added and incubated for 30 minutes. Following preincubation, the mixture was added to confluent cultures of L929 cells in 96 well microplates. The assay mixture, over a monolayer of L929 cells, contained antibody at concentrations of 0.001-10 μg/ml, recombinant human OPG at 0.1 μg/ml, actinomycin D at 1 μg/ml, and cross-linked human TRAIL at 20 ng/ml in a total volume of 150 μg/ml. The plate was incubated at 37 °C for 24 hours in a humidified CO_2 incubator. The medium was removed, the cells were fixed with 5% formaldehyde, and then stained with crystal violet. After dissolving the dye with 33% acetic acid, the optical density was read at 540 nm.

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.

ND_{50}: a working concentration of 0.2-0.7 μg/ml of Anti-OPG will neutralize 50% of the bioactivity due to 0.1 μg/ml recombinant human OPG using the TRAIL sensitive mouse L929 cytolytic assays.

Indirect immunoblotting: a working concentration of 0.1-0.2 μg/ml is determined using human OPG at 5 ng/lane under non-reducing conditions and 25 ng/ml under reducing conditions.

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

Endotoxin level is <10 ng per mg antibody as determined by the LAL method.

References

1. Kong, Y.Y. et al., Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature*, **402**, 304-309 (1999).
2. Hofbauer, L.C. et al., Stimulation of osteoprotegerin ligand and inhibition of osteoprotegerin production by glucocorticoids in human osteoblastic lineage cells: potential paracrine mechanisms of glucocorticoid-induced osteoporosis. *Endocrino*l., **140**, 4382-4389 (1999).
3. Hofbauer, L.C. et al., Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. *Endocrino*l., **140**, 4367-4370 (1999).
4. Kong, Y.Y. et al., OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature, **397**, 315-323 (1999).
5. Wong, B.R. et al., TRANCE is a TNF family member that regulates dendritic cell and osteoclast function. *J. Leukocyte Biol*., **65**, 715-724 (1999).
6. Anderson, D.M. et al., A homologue of the TNF receptor and its ligand enhance T-cell growth and dentritic-cell function. *Nature,* **390**, 175-179 (1997).
7. Ware, C. et al., Tumor necrosis factor-related ligands and receptors, in The Cytokine Handbook, 3rd Edition, Thomson, A.W., ed., Academic Press (San Diego, CA: 1998), pp. 549-592.
8. Tomoyasu, A., et al., Characterization of monomeric and homodimeric forms of osteoclastogenesis inhibitory factor. *Biochem. Biophys. Res. Comm*., **425**, 382-387 (1998).
9. Gravestein, L., and Borst, J., T., Tumor necrosis factor family members in the immune system. *Sem. Immunol*., **10**, 423-434 (1998).

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