

Product Information

Anti-AOP1 antibody, Mouse monoclonal
clone AOP-38, purified from hybridoma cell culture

Product Number **A7674**

Product Description

Anti-AOP1 antibody, Mouse monoclonal, (Antioxidant-like Protein 1) (mouse IgG1 isotype) is derived from the AOP-38 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to C-terminal amino acids 236-256 of human Aop1, conjugated to KLH. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2.

Monoclonal Anti-Aop1 reacts specifically with Aop1 and does not recognize other proteins involved in defense of oxidative stress, such as alkylhydroperoxide reductase (AhpC), glutathione peroxidase and peroxiredoxin. The epitope recognized by the antibody resides within the C-terminal amino acids 236-256 of human Aop1. The antibody may be used in ELISA and immunoblotting (25 kDa). Reactivity has been observed with human, monkey, dog, hamster, rat, mouse and chicken Aop1.

Living organisms produce reactive oxygen species such as H₂O₂ during physiological processes, and in response to external stimuli, such as UV radiation. To protect themselves against oxidative attacks, but also to maintain a redox balance in their different subcellular compartments, cells have evolved complex mechanisms.^{1,2} These antioxidant defense systems include nonenzymatic antioxidants (vitamin E, vitamin C, vitamin A, and uric acid), enzymes with antioxidant properties (catalase, superoxid dismutase, and glutathione peroxidase) as well as low molecular weight reducing agents (glutathione and thioredoxin).³ Oxidants and antioxidants represent a set of signaling molecules that modify function through redox. Antioxidants govern intracellular redox status. Similar to phosphorylation, redox can serve as the critical switch in many processes. Biologically relevant oxidants (e.g. hydrogen peroxide and nitric oxide) that serve as pleiotropic signaling molecules have been well documented.⁴ Balancing these oxidants are antioxidants such as glutathione, thioredoxin, and glutaredoxin. Thioredoxin reductase (TR), thioredoxin (Trx) and thioredoxin peroxidase (Tpx) are three linked

components in a redox chain that couples peroxide reduction to NADPH oxidation. In such a scheme within cells, Tpx is the immediate enzyme that detoxifies hydrogen peroxide, by reduction of H₂O₂ with the use of electrons provided by thioredoxin (Trx). Thioredoxin peroxidases are highly conserved in eukaryotes and prokaryotes.⁵ TPx exists as a dimer of identical 25 kDa subunits that contain 2 cysteine residues, Cys47 and Cys170. Cys47-SH appears to be the site of oxidation by peroxides, and the oxidized Cys47 probably reacts with Cys170-SH of the other subunit to form an intermolecular disulfide.⁶ The TPx disulfide is specifically reduced by thioredoxin, but can also be reduced (less effectively) by a small molecular size thiol.⁷ Members of the family of peroxiredoxin (Prx) proteins show amino acid sequence homology to TPx. More than 40 members of the Prx family have been identified in a wide variety of organisms ranging from prokaryotes to mammals.^{8,9} The ubiquity and structural conservation of peroxiredoxins suggest that they serve fundamentally important functions. The Prx family has been identified in association with a variety of diverse cellular functions including growth control, proliferation, differentiation, immune response, tumor promotion, and apoptosis, as well as activation of viruses (notably HIV, from latency). The mammalian Prx members (at least 12 proteins) can be divided into at least three distinct groups (Prx I, II, and III), on the basis of their amino acid sequences and immunological properties. Prx I and II are cytosolic proteins, whereas Prx III is localized in mitochondria. Human peroxiredoxins share 60 to 80% sequence identity to each other and more than 90% identity with the corresponding mouse homolog. AOE372 represents the prototype for a possible fourth subfamily (IV).² A novel member of the mammalian peroxiredoxin family, called AOEB166 (17 kDa) with mitochondrial and peroxisomal sorting signals, has also been described.³ The delicate interplay inside cells between oxidants and antioxidants ultimately determines the activity profile for many transcription factors.¹⁰ One redox regulated protein is NF-κB, a member of the Rel family of transcription factors that exist ambiently in the cytoplasm via association with inhibitor protein IκB.

A wide variety of stimuli, including tumor necrosis factor- α (TNF- α), phorbol ester, bacterial lipopolysaccharide, and virus infection can activate NF- κ B. Studies have implicated reactive oxygen species (*i.e.* H₂O₂) as one common signal transducer for these diverse stimuli.^{11,12} Aop1 (antioxidant-like protein 1, also called MER5, M.W. 25 kDa) a member of the family of proteins involved in defense of oxidative stress, seems to be involved in proliferation and differentiation, through antioxidant function and redox regulation.^{13,14} Monoclonal antibodies reacting specifically with Aop1 are useful tools for the determination of the functional activity of Aop1.

Reagents

Supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody Concentration: Approx. 2 mg/ml.

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.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of 0.25-0.5 μ g/ml is determined using a whole extract of cultured human acute T cell leukemia, Jurkat cells.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

1. Scandalios, J.G., *Oxidative Stress and the Molecular Biology of Antioxidant Defenses*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1997).
2. Jin, D.-Y., et al, *J. Biol. Chem.*, **272**, 30952-30961 (1997).
3. Knoops, B., et al., *J. Biol. Chem.*, **274**, 30451-30458 (1999).
4. Irani, K., et al., *Science*, **275**, 1649-1652 (1997).
5. Iwhara, S., et al., *Biochemistry*, **34**, 13398-13406 (1995).
6. Rhee, S.G., et al., *Biofactors*, **10**, 207-209 (1999).
7. Chae, H., et al., *J. Biol. Chem.*, **269**, 27670-27678 (1994).
8. Kang, S.W., et al., *J. Biol. Chem.*, **273**, 6297-6302 (1998).
9. Kang, S.W., et al., *J. Biol. Chem.*, **273**, 6303-6311 (1998).
10. Sen, C.K., and Packer, L., *FASEB J.*, **10**, 709-720 (1996).
11. Baeuerle, P.A., and Henkel, T., *Annu. Rev. Immunol.*, **12**, 141-179 (1994).
12. Schenk, H., et al., *Proc. Natl. Acad. Sci. USA*, **91**, 1672-1676 (1994).
13. Tsuji, K., et al., *Biochem. J.*, **307**, 377-381 (1995).
14. Jäschke, A., et al., *J. Mol. Biol.*, **277**, 763-769 (1998).

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