

Product Information

ANTI-NITROTYROSINE

Developed in Rabbit, Affinity Isolated Antibody

Product Number **N 0409**

Product Description

Anti-Nitrotyrosine is developed in rabbit using nitrated KLH as immunogen. Anti-Nitrotyrosine is affinity-purified using 3-nitro-L-tyrosine immobilized on agarose.

Anti-Nitrotyrosine recognizes nitrated proteins and 3-nitro-L-tyrosine. The antibody specifically recognizes nitrated BSA by immunoblotting (68 kD). The specificity of the antibody is determined by ELISA using nitrated BSA and 3-nitro-L-tyrosine-BSA conjugate. Anti-Nitrotyrosine does not cross-react with L-tyrosine, p-nitro-L-phenylalanine, 3-amino-L-tyrosine, 3-chloro-L-tyrosine and phospho-L-tyrosine BSA conjugates. Applications include the detection and localization of nitrated proteins by immunoblotting, ELISA, immunohistochemistry and immunofluorescence. Staining with anti-Nitrotyrosine by immunohistochemistry is specifically inhibited with 3-nitro-L-tyrosine.

Peroxynitrite (ONOO⁻), a potent oxidant linked to cellular oxidative damage, is an important member of the family of reactive oxygen and nitrogen species, rapidly generated *in vivo* from nitric oxide (NO[•]) and superoxide anion (O^{2-•}).^{1,2} The production of peroxynitrite *in vivo* has been demonstrated in the macrophage immune response and under conditions of oxidative stress such as ischemia-reperfusion.^{3,4}

Peroxynitrite can react with a wide range of biological molecules, including proteins, lipids and nucleic acids. Peroxynitrite has been shown to promote the nitration of tyrosine residues in proteins,⁵ and oxidation of redox metal centers⁶, DNA⁷, lipids⁸, and cysteine and methionine residues in proteins, suggesting that it plays a major role in oxidative cellular damage. Peroxynitrite has been implicated in the pathogenesis of several inflammatory, infectious and degenerative human disease^{9,10}, including neurological conditions such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS), in the pathogenesis of asthma, atherosclerosis and a variety of conditions precipitated by endothelial injury.¹¹⁻¹⁴ In light of its highly reactive nature, evidence for a role of peroxynitrite *in vivo* is largely based on the detection of 3-nitrotyrosine, the major product of peroxynitrite reaction, in injured tissues.^{9,12,14} Peroxynitrite reacts with superoxide dismutase (SOD) or transition metals to form nitronium-like reactive

species, which in turn nitrate tyrosine to form 3-nitrotyrosine in tissue proteins.⁵ In addition, a metabolite generally reflecting *in vivo* generation of peroxynitrite, is the modified amino acid 3-nitrotyrosine. Nitration of active-site tyrosines is known to compromise protein structure and function. Nitration of tyrosine has been shown to block tyrosine phosphorylation, a key event in signal transduction cascades, suggesting a role for peroxynitrite as a signaling molecule.¹⁵ Post-translational incorporation of nitrotyrosine in α -tubulin has been shown to cause microtubule dysfunction and alterations of cell morphology.¹⁶

Reagents

Anti-Nitrotyrosine is supplied as an affinity isolated antibody in 10 mM phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS 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 is not recommended. 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

A minimum working dilution of 1:1,000 is determined by immunoblotting using nitrated BSA.

A minimum working dilution of 1:1,000 is determined by ELISA using nitrated BSA and 3-nitro-L-tyrosine.

A minimum working dilution of 1:1,000 is determined by immunohistochemistry using methacarn-fixed, paraffin sections of Alzheimer's Disease brain.

A minimum working dilution of 1:1,000 is determined by immunofluorescence using methanol-fixed, cultured A549 cells metabolically labeled with 3-nitro-L-tyrosine.

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

References

1. Beckman, J.S., and Koppenol, W.H., *Am. J. Physiol.*, **271**, C1424 (1996).
2. Huie, R.E., and Padmaja, S., *Free Radical Res. Commun.*, **18**, 195 (1993).
3. Ischiropoulos, H., et al. *Arch. Biochem. Biophys.*, **298**, 446 (1992).
4. Beckman, J.S., et al., *Proc. Natl. Acad. Sci. USA*, **87**, 1620 (1990).
5. Ischiropoulos, H., et al. *Arch. Biochem. Biophys.*, **298**, 431 (1992).
6. Castro, L., et al., *J. Biol. Chem.*, **269**, 29409 (1994).
7. King, P.A., et al., *Nucleic Acids Res.*, **21**, 2473 (1993).
8. Radi, R., et al. *Arch. Biochem. Biophys.*, **288**, 481 (1991).
9. Ischiropoulos, H., et al. *Arch. Biochem. Biophys.*, **356**, 1 (1998).
10. Beckman, J.S., et al., *Nature*, **364**, 584 (1993).
11. Beckman, J.S., et al., *Biol. Chem. Hoppe-Seyler*, **375**, 81 (1994).
12. Smith, M.A., et al., *J. Neuroscience*, **17**, 2653, (1997).
13. White, C.R., et al., *Proc. Natl. Acad. Sci. USA*, **91**, 1044 (1994).
14. Buttery, L.D.K., et al., *Lab. Investig.*, **75**, 77, (1996).
15. Kong, S.K., et al., *Proc. Natl. Acad. Sci. USA*, **93**, 3377 (1996).
16. Eiserich, J.P., et al., *Proc. Natl. Acad. Sci. USA*, **96**, 6365 (1999).

lpg 5/00

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.