

Product Information

MONOCLONAL ANTI-NITRIC OXIDE SYNTHASE, INDUCIBLE (iNOS), CLONE NOS-IN

Mouse Ascites Fluid

Product Number **N 9657**

Product Description

Monoclonal Anti-Nitric Oxide Synthase, Inducible (iNOS) (mouse IgG1 isotype) is derived from the NOS-IN hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized BALB/c mouse. A synthetic peptide corresponding to nitric oxide synthase (NOS) of mouse macrophage origin (iNOS, also termed macNOS, amino acids 1126-1144) conjugated to KLH was used as the immunogen. The isotype is determined using Sigma ImmunoType Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Nitric Oxide Synthase, Inducible (iNOS) may be used for the localization of inducible-type nitric oxide synthase (iNOS), using various immunochemical assays such as ELISA, immunoblot, and immunocytochemistry.

Nitric oxide synthase (NOS) is an enzyme involved in the synthesis of nitric oxide (NO), a free radical generated under physiological conditions by virtually all mammalian cells.¹⁻³ NO is formed from arginine by NOS which oxidizes a guanidino nitrogen of arginine, releasing NO and citrulline. NO is a messenger molecule mediating diverse functions including vasodilatation, neurotransmission, and antimicrobial and antitumor activities. In addition, NO has been implicated as a pathogenic mediator in a variety of conditions, such as central nervous system (CNS) disease states. These include the animal model of multiple sclerosis (MS) and experimental allergic encephalomyelitis.⁴ The proteins predicted from the cDNA sequences of NOS isoforms in all species investigated, contain consensus sequences for the binding of NADPH, flavins and calmodulin. The C-terminal half of NOS possesses a high level of homology with NADPH-cytochrome P-450 reductase, where the predicted sites for binding NADPH and flavins are also located. However, the predicted heme and calmodulin binding sites of NOS are located within its N-terminal half. NOS has been localized in many different cell types. On the basis of molecular mass, subcellular location, and Ca²⁺ dependence, at least

three types of NOS have been classified. Type I NOS is found in neurons. It is a 150-160 kD protein, also called NOS-1, neuronal NOS (nNOS), brain NOS (bNOS), cerebral NOS, constitutive NOS or Ca²⁺-regulated NOS (cNOS). Type II, best characterized in macrophages, is a 130 kDa protein, also known as macrophage NOS (mNOS) or inducible NOS (iNOS). Type III is found in endothelial cells. It is a 135 kDa protein, also called endothelial NOS (eNOS, or ecNOS). Neuron and endothelial NOS are constitutively expressed and are dependent on Ca²⁺/calmodulin for NO production, whereas Type II NOS is Ca²⁺-independent and is expressed in activated macrophages and some glial cells after stimulation. Nevertheless, evidence indicates that the various types of NOS may serve a variety of diverse biological pathways.^{1,5,6} For instance, iNOS is not found only in macrophages but also in several other cell types including hepatocytes, chondrocytes, endothelial cells and fibroblasts. eNOS is not restricted to the endothelium of blood vessels but exists in the epithelium of several tissues, including the bronchial tree. It has also been localized to neurons in the brain, especially the pyramidal cells of the hippocampus, where it may function in long-term potentiation. bNOS is present also in skeletal muscle, where it is complexed with dystrophin, and is absent in Duchenne's muscular dystrophy, which perhaps accounts for symptoms of the disease.⁶ In addition, NOS seems to be a highly conserved enzyme, between the various types (e.g. a 52% amino acid identity of human bNOS and eNOS), and between species (e.g. 93% a.a. identity that exists between the rat and human bNOS). The production of isoform-specific antibodies to NOS⁷ allows investigators to identify which isoforms are present in a specific cell or tissue. These antibodies are invaluable for elucidating the expression of these isozymes in a variety of biological systems from cells to whole animals.

Monoclonal Anti-Nitric Oxide Synthase, Inducible (iNOS), reacts specifically with inducible-type nitric oxide synthase (iNOS, also termed macNOS and mNOS). It does not react with NOS derived from brain

(bNOS) or with endothelial-type NOS (eNOS). The product recognizes an epitope residing within the C-terminal amino acids 1126-1144 of the mouse iNOS molecule. This sequence is highly conserved in rat iNOS and diverges from the human iNOS. The antibody may be used in ELISA, immunoblotting (130kDa) and immunocytochemical staining. The product reacts with mouse iNOS.

Reagents

The product is provided as ascites fluid with 15 mM sodium azide as a preservative.

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 hazardous 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.

Product Profile

A minimum working dilution of 1:1,000 is determined by immunoblotting using a whole cell extract of the mouse macrophage cell line RAW 264.7 activated with lipopolysaccharide (LPS) and interferon- γ .

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

References

1. Dinerman, J.L., et al., Proc. Natl. Acad. Sci. USA, **91**, 4214 (1994).
2. Bredt, D.S., and Snyder, S.H., Proc. Natl. Acad. Sci. USA, **87**, 682 (1990).
3. Stuehr, J.J., and Griffiths, O.W., in: "Advances in Enzymology and Related Areas of Molecular Biology", Meister, A., (Ed.), J. Wiley & Sons, New York, **65**, 287, (1992).
4. Bagasra, O., et al., Proc. Natl. Acad. Sci. USA., **92**, 12041 (1995).
5. Snyder, S.H., Nature, **372**, 504 (1994).
6. Snyder, S.H., Nature, **377**, 196 (1995).
7. Pollock, J.S., et al., Histochemical J., **27**, 738 (1995).

LPG/KMR 07/02

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.