

Product Information

MONOCLONAL ANTI-NITRIC OXIDE SYNTHASE-BRAIN (bNOS) Clone NOS-B1 Mouse Ascites Fluid

Product No. **N 2280**

Product Description

Monoclonal Anti-Nitric Oxide Synthase-Brain (bNOS) (mouse IgG1 isotype) is derived from the NOS-B1 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from immunized BALB/c mice. Recombinant neuronal NOS fragment (amino acids 1-181) from rat brain was used as immunogen.¹ 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-Brain (bNOS) reacts specifically with nitric oxide synthase (NOS), derived from brain (bNOS, 150-160 kDa and several breakdown products of lower M.W.). It does not react with NOS derived from macrophages (mNOS) and endothelial cells (eNOS). The antibody may be used in ELISA, immunoblotting, dot blot immunoassay and immunohistochemical staining of formalin-fixed, paraffin-embedded tissue (e.g., tongue) or paraformaldehyde perfusion-fixed brain tissue. The product reacts with human, goat, porcine and rat bNOS.

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.^{2,3} NO is a messenger molecule mediating diverse functions including vasodilation, 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, including the animal model of multiple sclerosis (MS) and experimental allergic encephalomyelitis.⁴ NO is formed from arginine by NOS which oxidizes a guanidino nitrogen of arginine, releasing NO and citrulline. 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 kDa 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 immunological stimulation. Evidence indicates that the various types of NOS may have multiple activities.^{1,5,6} For instance, iNOS not only occurs in macrophages but 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% amino acid identity that exists between the rat and human bNOS). The production of isoform-specific antibodies to NOS⁷ allow investigators to identify which isoform(s) is 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-Brain (bNOS) may be used for the detection and localization of bNOS using various immunochemical assays including ELISA, immunoblot, dot blot and immunohisto-chemistry.

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 hazards and safe handling practices.

Product Profile

A minimum titer of 1:3,000 is determined by indirect immunoblotting using rat cerebellum extract.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

Storage

For continuous use, store at 2-8 °C for a maximum of 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.

References

1. Dinerman, J., et al., Proc. Natl. Acad. Sci. USA, **91**, 4214 (1994).
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3. Stuehr, J., and Griffiths, O., In: Advances in Enzymology and Related Areas of Molecular Biology, Meister, A. (ed.), **65**, John Wiley & Sons, New York, p 127 (1992).
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5. Snyder, S., Nature, **372**, 504 (1994).
6. Snyder, S., Nature, **377**, 196 (1995).
7. Pollock, J., et al., Histochemical J., **27**, 738 (1995).

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