

## Product Information

### ANTI-NITRIC OXIDE SYNTHASE, UNIVERSAL (uNOS)

Developed in Rabbit, Affinity Isolated Antibody

Product Number **N-217**

#### Product Description

Anti-Nitric Oxide Synthase, Universal (uNOS) is developed in rabbit using a synthetic peptide (Asp-Gln-Lys-Arg-Tyr-His-Glu-Asp-Ile-Phe-Gly), derived from amino acids 1113-1122 with N-terminally added Asp, of mouse iNOS and nNOS as immunogen. This sequence is highly conserved among the different NOS isoforms. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-Nitric Oxide Synthase, Universal (uNOS) recognizes mouse iNOS; rat, bovine and pig nNOS; and pig and cow Enos by immunoblotting. In immunohistochemistry, the antibody stains NOS in human endothelial cells, macrophages and peripheral neural tract cells. The antibody may be used to localize and detect the three isoforms of nitric oxide synthase (NOS): neuronal or brain (nNOS or bNOS), endothelial (eNOS) and inducible (iNOS); can be used in immunoblotting and immunohistochemistry.

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.<sup>1-3</sup> 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 anti-tumor 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.<sup>4</sup> 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 Ca<sup>2+</sup> 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 Ca<sup>2+</sup>-regulated NOS (cNOS). Type II, best characterized in macrophages, is a 130 kD 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 Ca<sup>2+</sup>/calmodulin for NO production, whereas Type II NOS is Ca<sup>2+</sup>-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.<sup>1,5,6</sup> 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.<sup>6</sup> 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 NOS<sup>7</sup> 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.

## Reagents

Anti-Nitric Oxide Synthase, Universal (uNOS) is supplied as an affinity isolated antibody in phosphate buffered saline containing 1 mg/ml bovine serum albumin and 0.05% 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 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. Working dilution samples should be discarded if not used within 12 hours.

## Product Profile

A minimum working dilution of 1:1,000 is determined for immunoblotting and 1:100 for immunohistochemistry.

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

## References

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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).
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