

## Product Information

### Monoclonal Anti-Nitric Oxide Synthase, Universal clone NOS-3F7-B11-B5

produced in mouse, ascites fluid

Catalog Number **N218**

#### Product Description

Monoclonal Anti-Nitric Oxide Synthase, Universal is produced in mouse using bovine cerebellum NOS as immunogen.

Monoclonal Anti-Nitric Oxide Synthase, Universal reacts with iNOS, eNOS and nNOS from rat and bovine, and iNOS in induced RAW 264.7 mouse macrophages. 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) by immunoblotting and immunohistochemistry. In immunoblotting, the antibody detects a 130 kDa band representing iNOS in samples first induced with IFN (interferon) and LPS (lipopolysaccharides). It also detects a 155 kDa band in tissues expressing nNOS and a 140 kDa band in tissues expressing eNOS.

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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$ /calmodulin for NO production, whereas Type II NOS is  $\text{Ca}^{2+}$ -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.

**Reagent**

Supplied as mouse ascites fluid containing  $\leq 0.1\%$  sodium azide.

**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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.

**Product Profile**

Recommended working dilutions:  
1:50-1:500 for immunoblotting  
1:100 for immunohistochemistry (frozen).

**References**

1. Rengasamy, A., et al., "Immunohistochemical demonstration of a paracrine role of nitric oxide in bronchial function." *Am. J. Physiol.*, **267**, L704-L711 (1994).
2. Xue, C., et al., "Distribution of NOS in normoxic vs. hypoxic rat lung: upregulation of NOS by chronic hypoxia." *Am. J. Physiol.*, **267**, L667-L678 (1994).

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