ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Nitric Oxide Synthase (bNOS/iNOS/eNOS; NOS I-III) Pan Antibodies

Cat # NOSP51-M Mouse Monoclonal Anti-bovine brain NOS protein (NOS I-III, Pan), ascites **SIZE**: 100 ul

Nitric oxide (NO), a diffusible free radical gas, acts as a neurotransmitter in brain and peripheral nervous system. It accounts for the activity of endothelium-derived relaxing factors, which stimulate vasodilatation by releasing NO from the endothelium. NO is synthesized by L-arginine, oxygen, and NADPH by three known isoforms of heme-containing flavoproteins termed NO synthase (NOS, I-III, mol wt. ~130-160 kDa). One group of enzyme is constitutive, agonist-triggered, and dependent on Ca2+/Calmodulin and is inhibited by L-arginine analogues (L-NNA, L-NMMA). It is found in endothelium, adrenal glands, brain and platelets. The other principle group is inducible, Ca^{2+}/Calmodulin-independent, and inhibited by NMMA and L-NNA. It has been found in macrophage, hepatocytes, tumor cells, vascular smooth muscle and endothelial cells. Analyses of cDNA clones have identified three distinct NOS genes in mammals: neuronal (nNOS/bNOS/NOS-I), endothelial (eNOS/NOS-III), and macrophage (mNOS/iNOS/NOS-II). Both nNOS and eNOS are constitutive and the mNOS/iNOS is inducible. Sequence homology among different cloned isoforms is ~ 50%. Human, rat, mouse, and bovine eNOS/NOS-3 are ~1202-1205 aa proteins (1).

Protein name Nitric-oxide synthase, endothelial
Synonyms EC 1.14.13.39; EC-NOS; NOS type III; NOSIII; Endothelial NOS; eNOS; Constitutive NOS; cNOS ; Gene name Name: NOS3

Source of Peptide Antigen and Antibodies

Antigen	Highly purified bovine brain NOS protein used as antigen
Ab Host/type	Mouse, monoclonal ascites (IgM isotype), (Cat # eNOS51-M) supplied in PBS+0.05% azide
2-Ab	**Goat Anti-mouse IgG (H+l)-HRP conjugate Cat # 40320 or anti-mouse IgM-HRP conjugate (#40220)**
-Ve control	Cat # 20102-105, Control mouse IgM (non-immune) can be used –ve control in ELISA, Western or IHC

Affinity pure IgG

100 ug/100ul solution	50 ug/50 ul lyophilized powder

Supplied in Buffer: PBS, pH 7.5, 0.1% BSA, 0.05% azide
Reconstitute powder in PBS at 1 mg/ml

Storage

Short-term: unopened, undiluted vials for less than a week at 4oC.

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.

Recommended Usage

Western Blotting. (1-10 ug/ml for affinity pure). The solution should be diluted 1:1K or more before use. The antibody recognizes 135-140 kDa protein bovine recombinant protein control (#eNOS51-C) or mouse, human eNOS proteins. see refs 2.

ELISA: Control peptide should be coated at 1 ug/ml.

Immunocytochemistry. We recommend the use of affinity pure antibody to reduce background (use at 5-10 ug/ml). Useful on tissue sections fixed with 3.5% paraformaldehyde. see refs 2.

Specificity and Cross-reactivity

NOS isoforms (NOSI-III) show significant sequence conservation across the species. This antibody epitope appears to be a conservative region among various isoforms. Antibody produced to purified bovine brain NOS protein (cat# NOSP51-M) 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 ~155 Kda), endothelial (eNOS ~140 Kda) and inducible (iNOS or mNOS ~130 Kda) by immunoblotting and immunohistochemistry. Therefore this antibodies in designated Pan or reactive with the 3 NOS isoforms. Several recombinant purified proteins for human bNOS (#bNOS11-C), rat bNOS14-C, mouse iNOS (#iNOS-C), bovine enOS (#eNOS32-C), and human eNOS (#eNOS-C) can be used as positive controls for Western blot.

General References: (1) MARSDEN P.A FEBS LETT. 307, 287-293 (1992); JANSSENS S.P., J. BIOL. CHEM. 267, 14519-14522 (1992); Michel T (1992) J Card. Pharmacol.20, S45-S49

(2) Citations of ADI's eNOS Antibodies (see web site for updates)
El-Omar MM, 2003 J. Heart Failure 5, 229-239 WB,
Ulbrich SE, 2006, J. Endocrinol.,188: 205 – 213, IHC
Dorenkamp M, 2005, Eur. J. Pharmacol. 520, 27 179, 187, IHC
Bianca DV, 2006, J. Pharmacol. Exp. Ther.,316: 703 - 708. WB

For In Vitro Research Use and Manufacturing Only.

NOSP51-M 71122A

Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, San Antonio, TX 78244, USA; **Email**: service@4adi.com
☎ (800) 786-5777; (210) 561-9515; **Fax**: (210) 561-9544; **Web Site**: www.4adi.com