ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Nitric Oxide Synthase 1 (bNOSn/NOS-1) Antibodies

Cat # bNOS12-M Mouse Monoclonal Anti-rat bNOS antibody, **SIZE**: 100 ul (**neat ascites**)

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. Unlike typical neurotransmitter, NO is a not stored in synaptic vesicle and does not act on membrane receptors. Synthesis of NO, initially demonstrated in vascular endothelium, is now found in many tissues.

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, and mouse bNOS/NOS-1 are ~1433 aa, 1429 aa, and 1429 aa protein respectively (1).

Source of Peptide Antigen and Antibodies

Antigen	amino terminal region 1-180 aa fusionprotein of rat brain NOS-1
Ab Host/type	Balb/c **mouse**. Splenocytes were fused with Sp2/0 myeloma cells. Resulting clone (designated NOS1, isotype IgG1), selected for reactivity with bNOS, was expanded into mice as **ascites**. Antibody has been purified by Protein A/G column chromatography.
2-Ab	**Goat Anti-mouse IgG-HRP conjugate Cat # 40320 (AP, biotin, FITC conjugates also available)**)
-ve control IgG	Cat # 20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form and Storage of Peptide and Antibodies

Ascites (unpurified, undiluted)

100 ul/vial

solution lyophilized powder

contains 0.05% sodium azide

Reconstitute powder in the original vol. of water

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.

Shipping: 4oC for solutions and room temp for powder.

Recommended Usage

Western Blotting. Ascites should be diluted 1:1K or more before use. It is suggested that user optimize actual dilution and conditions according their application. The antibody recognizes 130 kDa protein in Western blots.

ELISA: Control anigen 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. An overnight incubation with antibody at 4oC is recommended and detection by ABC (peroxidase) technique.

Cross-reactivity

Antibody crossreacts with mouse, rat, and human iNOS. No significant reactivity is seen with NOS-2/NOS-3. Antibody cross-reactivity in various species has not been studied.

General References:

Bredt DS et al (1991) Nature 351, 714-718; Nakane M (1993) FEBS Lett. 316, 175-180.

For In Vitro Research Use and Manufacturing Only.

Related material available from ADI

Anti-bNOS (NOS I) Anti-iNOS (NOS II), Anti-eNOS (NOS III) Antibodies and Control Peptide

Purified/Recombinant NOSs as Positive Controls.

Western Blot recycling kit (strip antibodies in 5-10 at room temp; No heating or mercaptoethanol).

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