ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Nitric Oxide Synthase I (bNOS/nNOS/NOS-I) Antibodies

Cat # bNOS14-P	Rat bNOS/NOS-1 Control/blocking Peptide # 4	**SIZE**: 100 ug
Cat # bNOS14-S	**Chicken** Anti-Rat bNOS/NOS-1 antiserum # 4	**SIZE**: 100 ul
Cat # bNOS14-C	**Rat** bNOS/NOS-1 purified protein WB +ve control	**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. 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%.

Source of Peptide Antigen and Antibodies

Human, rat, and mouse bNOS/NOS-1 are 1433 aa, 1429 aa, and 1429 aa proteins respectively (1). An Amino acid sequence corresponding to rat brain NOS (**designated bNOS14-P; control peptide**; 1409-1429 aa) (1) was synthesized and coupled to KLH and antibodies produced in **chicken**.

Rat nNOS was expressed in baculovirus (Sf9) cells and purified (~95%) using column chromatography. Rat Brain NOS protein WB +ve control **(#bNOS14-C**) is supplied in 100 ul SDS-PAGE sample buffer (reduced). Rat bNOS will give ~150 kDa band when detected with appropriate antibodies. It should be stored frozen at -20oC or lower in small aliquots. It should be heated once prior to loading on gels. Repeated heating or freezing and thawing is not recommended.

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied 0.05% azide, **Reconstitute** powder in 100 ul PBS

Control/blocking peptide
100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage

Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

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. 1:1K or more for antiserum. It is suggested that user optimize actual dilution and conditions according their application. The antibody recognizes ~150-160 kDa protein in Western blots.

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

Immunocytochemistry. Not tested**.**

Antibody specificity and Cross-reactivity

Rat bNOS14-P peptide sequence is conserved in rabbit (95%), human (95%), and mouse (95%). No significant sequence homology of bNOS14-P is seen with NOS-2/NOS-3 or other proteins. Antibody crossreactivity in various species is not established. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol see detailed protocol at the web site).

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

For In Vitro Research Use and Manufacturing Only.

Related material available from ADI

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

BNOS14-S-P-C 71212A

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