ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

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

Cat # bNOS13-P	bNOS/NOS-1 Control Peptide # 3	**SIZE**: 100 ug
Cat # bNOS13-S	Rabbit Anti-bNOS/NOS-1 antiserum # 3,	**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%. Human, rat, and mouse bNOS/NOS-1 are 1433 aa, 1429 aa, and 1429 aa proteins respectively (1).

Source of Peptide Antigen and Antibodies

Antigen	An Amino acid sequence corresponding to rat brain NOS (**designated bNOS13-P; control peptide**; 251-270 aa) (1) was synthesized and coupled to KLH
Ab Host/type	Rabbit, Polyclonal antiserum # **bNOS13**-S
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control IgG	Cat # 20009-1, Rabbit (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

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 Neat serum and 1-10 ug/ml for affinity pure). solution 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 ~150-160 kDa protein in Western blots.

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

Cross-reactivity

Rat bNOS13-P peptide sequence is conserved in mouse (85%), human (84%), and rabbit (68%). No significant sequence homology of bNOS13-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

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

Purified/Recombinant NOSs as Positive Controls.

bNOS-S-P 71212A

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