ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

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

Cat # bNOS-P	bNOS/NOS-1 Control Peptide	**SIZE**: 100 ug
Cat # bNOS-S	Rabbit Anti-bNOS/NOS-1 antiserum # 1,	**SIZE**: 100 ul
Cat # bNOS-A	Rabbit Anti-bNOS/NOS-1 Ab # 1 Aff pure,	**SIZE**: 100 ug
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. 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). Sequence homology among different isoforms is ~ 50%.

Source of Peptide Antigen and Antibodies

Antigen	An Amino acid sequence corresponding to human brain NOS (designated bNOS-P; control peptide; 724-739 aa) (1) was synthesized and coupled to KLH
Ab Host/type	Rabbit, Polyclonal antiserum # bNOS -S and IgG, purified over antigen-agarose (Cat # bNOS -A)
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

Rat nNOS/bNOS protein for Western blot +ve control (**Cat # bNOS14-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **bNOS14-C** for good visibility with antibody Cat # **bNOS-S**. Store at –20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the **bNOS14-C** solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. This preparation is intended for qualitative purpose and not to serve as standard of known concentration. Do not freeze, thaw, or hear repeatedly

Form & Storage of Antibodies/Peptide Control

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

Affinity pure IgG
100 ug/100ul solution lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

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). It is suggested that user optimize actual dilution and conditions according their application.

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.

Cross-reactivity

Human bNOS-P peptide sequence is 100% conserved in mouse, rat, and rabbit. No significant sequence homology of bNOS-P is seen with NOS-2/NOS-3 or other proteins. Antibody cross-reactivity in various species has not been studied. 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.

bNOS-S-A-P-14-C 71212A

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