

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 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, Ca²⁺/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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