

Product Specification Sheet

Nitric Oxide Synthase I (bNOS/nNOS/iNOS) 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 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%.

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 -200C 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).

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