

Product Specification Sheet

Nitric Oxide Synthase 3 (eNOS/NOS-3) Antibodies

Cat # eNOS32-P	Mouse eNOS/NOS-3 Control Peptide # 2	SIZE: 100 ug
Cat # eNOS32-A	Rabbit Anti-Mouse eNOS/NOS-3 IgG # 2, aff pure	SIZE: 100 ug

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

Antigen	20-aa peptide of mouse eNOS (1); Designated (eNOS32-P or control peptide) coupled to KLH; epitope location ~ C-terminus
Ab Host/type	Rabbit, Polyclonal IgG, purified over antigen-agarose (Cat # PHD11-A) supplied in PBS+0.1% BSA+0.05% azide
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-Ve control	Cat # 20009-1, Control Rabbit (non-immune) Serum IgG can be used -ve control in ELISA, Western or IHC

Form & Storage of Antibodies/Peptide Control

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. (1-10 ug/ml for affinity pure). The 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 135-140 kDa protein.

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

Mouse eNOS32-P peptide sequence is quite conserved in rat, human, bovine, and canine eNOS. No significant sequence homology of eNOS32-P is seen with NOS-1/NOS-2 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) MARSDEN P.A FEBS LETT. 307, 287-293 (1992); JANSSENS S.P., J. BIOL. CHEM. 267, 14519-14522 (1992); Michel T and Lamas, S (1992) J Card. Pharmacol.20, S45-S49

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

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

ENOS32-A-P 71211A

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