ALPHA DIAGNOSTIC INTERNATIONAL

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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 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%.

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).

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Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, S an Antonio, T X 7 8 24 4 , U S A;

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400 Fax: +91-11-42208444 Email: customerservice@lifetechindia.com Website: www.lifetechindia.com