ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

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

Cat # eNOS41-P	Bovine eNOS/NOS-3 Control Peptide	**SIZE**: 100 ug
Cat # eNOS41-M	Mouse Monoclonal Anti-bovine eNOS/NOS-3 ascites	**SIZE**: 100 ul
Cat # eNOS41-C	Recombinant purified Bovine eNOS/NOS-3 protein control for WB	**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. 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%. Human, rat, mouse, and bovine eNOS/NOS-3 are ~1202-1205 aa proteins (1).

Protein name Nitric-oxide synthase, endothelial
Synonyms EC 1.14.13.39; EC-NOS; NOS type III; NOSIII; Endothelial NOS; eNOS; Constitutive NOS; cNOS ; Gene name Name: NOS3

Source of Peptide Antigen and Antibodies

Antigen	A synthetic peptide (1185-1205) of bovine eNOS (protein accession #P29473, refs 1); **Designated (eNOS41-P or control peptide)** coupled to KLH; **Epitope location**~ C-terminal,
Ab Host/type	Mouse, monoclonal ascites (IgA isotype), (Cat # eNOS41-M) supplied in PBS+0.05% azide
2-Ab	**Goat Anti-mouse IgA-HRP conjugate** Cat # 40020 or goat anti-mouse Fab-HRP (AP, biotin, FITC conjugates also available)
-Ve control	Cat # 20102-100, Control mouse IgA (non-immune) can be used –ve control in ELISA, Western or IHC

Bovine eNOS/NOS-III (~135 Kda) was expressed in sf9 cells and purified (>95%). For Western blot +ve control (**Cat # eNOS41-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **eNOS41-C** for good visibility with antibody Cat # **eNOS41-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 **eNOS41-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

Affinity pure IgG

100 ug/100ul solution	50 ug/50 ul lyophilized powder

Supplied in Buffer: PBS, pH 7.5, 0.1% BSA, 0.05% azide
Reconstitute powder in PBS at 1 mg/ml

Control/blocking peptide

100 ug/100 ul solution	50 ug/50 ul lyophilized powder

Supplied in Buffer: PBS, pH 7.5 , 0.05% azide
Reconstitute powder in PBS at 1 mg/ml

Storage

Short-term: unopened, undiluted vials for less than a week at 4oC.

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.

Recommended Usage

Western Blotting. (1-10 ug/ml for affinity pure). The solution should be diluted 1:1K or more before use. The antibody recognizes 135-140 kDa protein bovine recombinant protein control (#eNOS41-C) or mouse, human eNOS proteins. see refs 2.

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. see refs 2.

Specificity and Cross-reactivity

Bovine eNOS41-P peptide sequence is 100% conserved in rabbit, pig, sheep, 93% in canine, chicken, human, and Chimp, 87% in mouse, 85% in rat NOS-3/eNOS/NOS-III proteins. No significant sequence homology of eNOS41-P is seen with NOS-1/NOS-2 or other proteins. Antibodies react with mouse, rat, human and human eNOS. 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. Bovine eNOS control protein #eNOS41-C or human eNOS (#eNOS-C) can be used as positive control for Western.

General References: (1) MARSDEN P.A FEBS LETT. 307, 287-293 (1992); JANSSENS S.P., J. BIOL. CHEM. 267, 14519-14522 (1992); Michel T (1992) J Card. Pharmacol.20, S45-S49

(2) Citations of ADI's eNOS Antibodies (see web site for updates)
El-Omar MM, 2003 J. Heart Failure 5, 229-239 WB,
Ulbrich SE, 2006, J. Endocrinol.,188: 205 – 213, IHC
Dorenkamp M, 2005, Eur. J. Pharmacol. 520, 27 179, 187, IHC
Bianca DV, 2006, J. Pharmacol. Exp. Ther.,316: 703 - 708. WB

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