ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

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

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| Cat # eNOS-P | Human eNOS/NOS-3 Control Peptide # 1 | **SIZE**: 100 ug |
| Cat # eNOS-C | Recombinant Human eNOS/NOS-3 protein control for WB | **SIZE**: 100 ul |
| Cat # eNOS-S | Rabbit Anti-Human eNOS/NOS-3 antiserum # 1, | **SIZE**: 100 ul |
| Cat # eNOS-A | Rabbit Anti-Human eNOS/NOS-3 IgG # 1, 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. 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).

## Source of Peptide Antigen and Antibodies

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| **Antigen** | A 15 amino acid peptide sequence corresponding to human eNOS **(designated eNOS-P; control peptide**) (1) was synthesized and coupled to KLH |
| **Ab Host/type** | Rabbit, Polyclonal antiserum # **eNOS** -S and IgG, purified over antigen-agarose (Cat # **eNOS** -A) |
| **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 |

Human e-NOS (protein accession #P29474, ~130 Kda) was expressed in sf9 cells and partically purified. For Western blot +ve control (**Cat # ENOS-C**) is obtained from 50,000xg supernatant and supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **ENOS**-C for good visibility with antibody Cat # **ENOS**-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 **ENOS**-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. Do not freeze and thaw, or heat repeatedly.

## Form & Storage of Antibodies/Peptide Control

**Antiserum (unpurified)**

100ul solution lyophilized powder

Supplied in Buffer: 0.05% azide

**Reconstitute** powder in 100 ul PBS

**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**. (1:1K –5K for antiserum and 1-10 ug/ml for affinity pure). ENos ~135-140 kDa protein (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). See refs 2.

## Specificity and Cross-reactivity

Human eNOS-P peptide sequence is 100% conserved in mouse/bovine, 93% in rat and 86% in pig. No significant sequence homology of eNOS-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**: 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

**Citations of for eNOS (**see updated list at the web site)

Ulbrich SE, 2006, J. Endocrinol., Feb 2006; 188: 205 – 213, IHC

Dorenkamp M, 2005, Eur. J. Pharmacology, 520, 179-187, , IHC

Bianca DV, 2006, J. Pharmacol. Exp. Ther.,316: 703 - 708., WB,

El-Omar MM, 2003, J. Heart Failure 5, 229-239, WB,,

Ahmed A, 2005, Fetal & Pediatric Pathol. 2005, 3, p141-147,, , IHC

For In Vitro Research Use and Manufacturing Only.

**Related material available from ADI**

Anti-NOSI-III, Purified/Recombinant NOSs

eNOS-S-A-P-C 80611A

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