ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Inducible Nitric Oxide Synthase 2 (iNOS/NOS-2) Antibodies

Cat # iNOS-P	Mouse iNOS/NOS-2 Control Peptide # 1	**SIZE**: 100 ug
Cat # iNOS-S	Rabbit Anti-Mouse iNOS/NOS-2 antiserum # 1,	**SIZE**: 100 ul
Cat # iNOS-A	Rabbit Anti-Mouse iNOS/NOS-2 IgG# 1, aff pure	**SIZE**: 100 ug
Cat # iNOS-C	Purified Mouse iNOS protein Western blot +Ve control	**SIZE**: 100 ul

Nitric oxide (NO), a diffusible free radical gas, acts as a neurotransmitter in brain and peripheral nervous system. 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%. Human i-NOS (1153-aa) and mouse iNOS/NOS-2 (1144-aa) proteins. I-Human i-NOS has 2 known isoforms: isoform 1 (1153-aa) and isoform 2 (1114-aa; missing 264-288 and 298-311 aa;

Protein name Nitric oxide synthase, inducible
Synonyms EC 1.14.13.39; NOS type II; Inducible NO synthase Inducible NOS; iNOS; Macrophage NOS; MAC-NOS
Gene name Name: Nos2 ; Synonyms: Inosl

Source of Peptide Antigen and Antibodies

Antigen	14-aa peptide 1131-1144 of mouse I-NOS/NOS-2 (protein accession #, P29477, refs 1) ; **Designated (INOS-P or control peptide)** conjugated to KLH; Epitope ~CT
Ab Host/type	Rabbit, Polyclonal antiserum # INOS-S and IgG, purified over antigen-agarose (Cat # INOS-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (biotin, FITC conjugates)
-ve control IgG	Cat # 20009-1, Rabbit (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

Mouse i-NOS/NOS-2 is purified from activated murine macrophage cells (.95%). For Western blot +ve control **(Cat # iNOS-C)** is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of iNOS -C for good visibility with antibody Cat # iNOS-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 iNOS -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, thaw, or heat repeatedly.

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied in: 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 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.

Recommended Usage

Western Blotting. (1:1K-3K of neat serum or 1-10ug/ml of affinity pure IgG). The antibody recognizes 130 kDa protein in Western blots from mouse macrophage RAW264.7 cells. 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.

Antibody specificity and Cross-reactivity

Mouse iNOS-P peptide sequence is 85% conserved in human and rat iNOS. No significant sequence homology of iNOS-P is seen with NOS-1/NOS-3 or other proteins. Antibody crossreactivity in various other 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.

General References: (1) Lowenstein CJ et al (1992) PNAS 89, 6711; Lyons CR et al (1992) J Biol Chem. 267, 6370; Zie Q-W (1992) Science 256, 225; Nunokawa Y et al (1993) BBRC 191, 89

(2) Citations of ADI antibodies to iNOS
Xu, X et al, 2001, Biochemical Pharmacology, 59, 509-516; XU, X, 2001, European Journal of Pharmacology, 416, 1-9 WB, Grassellia, F.2001, Domestic Animal Endocrinol. 20, 241-252, WB

For In Vitro Research Use and Manufacturing Only.

INO-S-A-P-C 71121A

Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, San Antonio, TX 78244, USA; **Email**: service@4adi.com
☎ (800) 786-5777; (210) 561-9515; **Fax**: (210) 561-9544; **Web Site**: www.4adi.com