ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

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

Cat # INOS22-P	Mouse iNOS/NOS-2 Control Peptide # 2	**SIZE**: 100 ug
Cat # INOS22-S	Rabbit Anti-Mouse iNOS/NOS-2 antiserum # 2	**SIZE**: 100 ul
Cat # INOS25-C	Recombinant Mouse iNOS/NOS-2 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, and mouse iNOS/NOS-2 are ~1145 aa proteins (1).

Source of Peptide Antigen and Antibodies

Antigen	A 19 amino acid peptide sequence corresponding to mouse iNOS (**designated INOS22-P; control peptide**; 1125-1144 aa) (1) was synthesized, coupled to KLH
Ab Host/type	Rabbit, Polyclonal antiserum # **INOS22**-S
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

Mouse NOS2 protein (full length; gene accession # BC062378) was expressed as fusion protein (His tag-NOS2) in E.coli and purified (>95% with major band at ~130kDa). For Western blot +ve control (Cat # **INOS25**-C) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **INOS25**-C for good visibility with antibody Cat # **INOS25**-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 **INOS25**-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 0.05% azide, **Reconstitute** powder in 100 ul PBS

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-3K). It is suggested that users optimize actual dilution and conditions according their application. 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. An overnight incubation with antibody at 4oC is recommended and detection by ABC (peroxidase) technique. Positive staining is seen in Glial, Purkinje and layer 5 cortical neurons.

Antibody specificity and Cross-reactivity

Mouse iNOS22-P peptide sequence is 84% conserved in human and rat (16/19 aa;). 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 (see detailed protocol see detailed protocol at the web site).

General References: (1) Lowenstein CJ et al (1992) PNAS 89, 6711-6715; Lyons CR et al (1992) J Biol Chem. 267, 6370-6374; Zie Q-W et al (1992) Science 256, 225-228; Nunokawa Y et al (1993) BBRC 191, 89

Citations of for iNOS (see updated list at the web site)
XU, X, 2001, Eur. J. Pharmacol. 416, 1-9 (WB); Xu, X, 2001, Biochem. Pharmacol. 59, 509-516 (WB); Leiro JM, 2003, Int. J. Immunopharmacol. 4, 163-177; Leiro J, 2004, J. Leukoc. Biol., 75: 1156 – 1165;

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