ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

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

Cat # iNOS23-M	Mouse Monoclonal Anti-iNOS/NOS-2 peptide, ascites	**SIZE**: 100 ul
Cat # iNOS23-P	Mouse iNOS/NOS-2 control peptide # 3	**SIZE**: 100 ug
Cat # iNOS25-C	Recombinant purified Mouse iNOS/NOS-2 control protein for WB	**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	Human, rat, and mouse iNOS/NOS-2 are ~1145 aa proteins (1). A peptide sequence **(designated as INOS23-P, control peptide)** within the C-terminus of mouse iNOS (1) was selected for antibody production in mice.
Ab Host/type	Mouse, Monoclonal, ascites, IgG1, (**cat # INOS23-M)** in PBS, pH 7.5 containing 0.05% azide,
2-ab	Goat Anti-mouse IgG-HRP conjugate Cat # 40320 (AP, biotin, FITC conjugates also available)
-ve control IgG	Cat # 20008-1, Mouse (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 # **INOS23**-M. 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

Ascites (unpurified, undiluted)

100 ul/vial solution contains 0.05% sodium azide — lyophilized powder

Reconstitute powder in the original vol. of water

Control/blocking peptide

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

Buffer: PBS, pH 7.5 and 0.05% sodium azide

Reconstitute powder in the original vol. of water

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.
Shipping: 4oC for solutions and room temp for powder.

Recommended Usage

Western Blotting. Ascites 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 130 kDa mouse eNOS protein in Western blots.

ELISA: Control peptide should be coated at 1 ug/ml.

Immunocytochemistry. Not tested. 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.

Antibody specificity Cross-reactivity

Antigenic peptide #INOS23-P is 100% conserved in rat iNOS. Antibody crossreact with mouse, rat. No significant reactivity is seen with NOS-1/NOS-3. 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 at: www.4adi.com\data\abblock.html).

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, 89-94;

For In Vitro Research Use and Manufacturing Only.

Related material available from ADI
Anti-NOS I-III and Purified/Recombinant NOSs as Positive Controls.
INOS23-M-C 110801A

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