Anti-iNOS Antibody

ER1706-89

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Chicken
Applications:	IF-Cell, FC, WB, IHC-Fr
Molecular Wt:	Predicted band size: 131 kDa

Description: Nitric oxide (NO) has a broad range of biological activities and has been implicated in signaling pathways in phylogenetically diverse species. Nitric oxide synthases (NOSs), the enzymes responsible for synthesis of NO, contain an N-terminal oxygenase domain and a C-terminal reductase domain. NOS activity requires homodimerization as well as three cosubstrates (L-arginine, NADPH and O2) and five cofactors or prosthetic groups (FAD, FMN, calmodulin, tetrahydrobiopterin and heme). Several distinct NOS isoforms have been described and been shown to represent the products of three distinct genes. These include two constitutive Ca2+/CaM-dependent forms of NOS, including NOS1 (also designated ncNOS) whose activity was first identified in neurons, and NOS3 (also designated ecNOS), first identified in endothelial cells. The inducible form of NOS, NOS2 (also designated iNOS), is Ca2+-independent and is expressed in a broad range of cell types.

Immunogen: Synthetic peptide within human iNOS aa 1,104-1,153 / 1,153.

Positive control: RAW264.7 treated with 1μg/mL LPS for 24 hours whole cell lysate, RAW264.7 whole cell lysate, HeLa cell lysate, A549 cell lysate, A549, LOVO.

Subcellular location: Cytoskeleton, Nucleus, Cytosol.

Database links: SwissProt: P35228 Human | P29477 Mouse

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:200
FC	1:50-1:100
IHC-Fr	1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze/thaw cycles.

Purity: Immunogen affinity purified.

Images

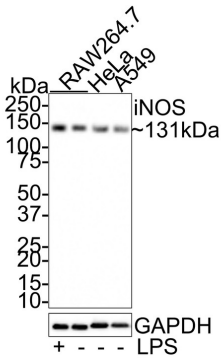

Fig1: Western blot analysis of iNOS on different lysates with Rabbit anti-iNOS antibody (ER1706-89) at 1/1,000 dilution.

Lane 1: RAW264.7 treated with 1µg/mL LPS for 24 hours whole cell lysate (20 µg/Lane)
Lane 2: RAW264.7 whole cell lysate (20 µg/Lane)
Lane 3: HeLa cell lysate (30 µg/Lane)
Lane 4: A549 cell lysate (30 µg/Lane)

Predicted band size: 131 kDa
Observed band size: 131 kDa

Exposure time: 16 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ER1706-89) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

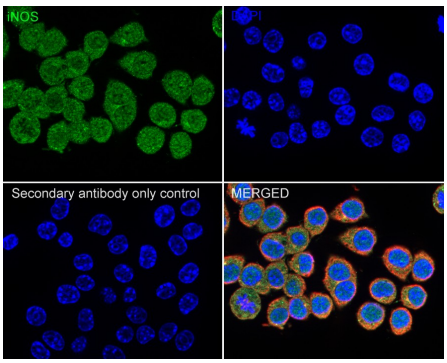

Fig2: Immunocytochemistry analysis of RAW264.7 cells labeling iNOS with Rabbit anti-iNOS antibody (ER1706-89) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-iNOS antibody (ER1706-89) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

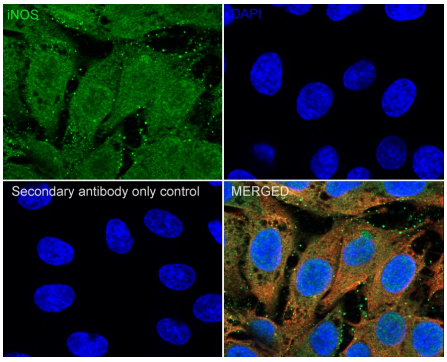

Fig3: Immunocytochemistry analysis of C6 cells labeling iNOS with Rabbit anti-iNOS antibody (ER1706-89) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-iNOS antibody (ER1706-89) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

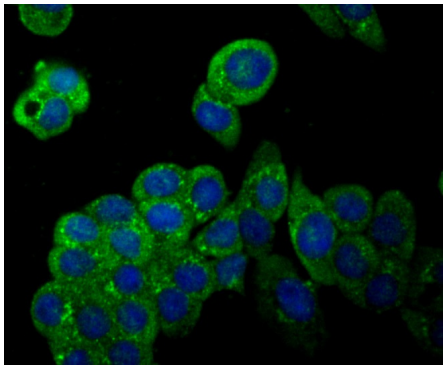

Fig4: ICC staining iNOS in LOVO cells (green). The nuclear counter stain is DAPI (blue). Cells were fixed in paraformaldehyde, permeabilised with 0.25% Triton X100/PBS.

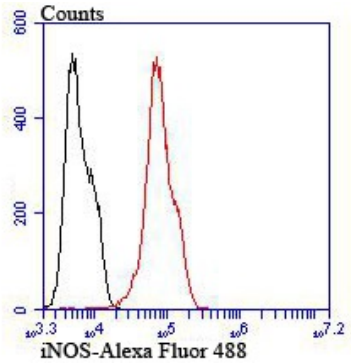

Fig5: Flow cytometric analysis of LOVO cells with iNOS antibody at 1/100 dilution (red) compared with an unlabelled control (cells without incubation with primary antibody; black).

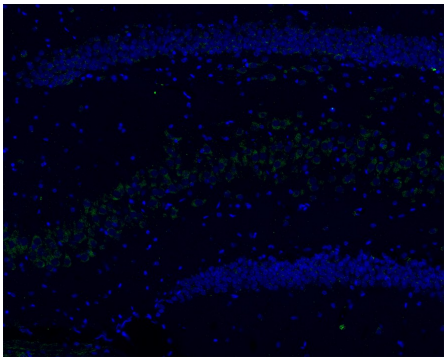

Fig6: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling iNOS with Rabbit anti-iNOS antibody (ER1706-89). The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ER1706-89, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

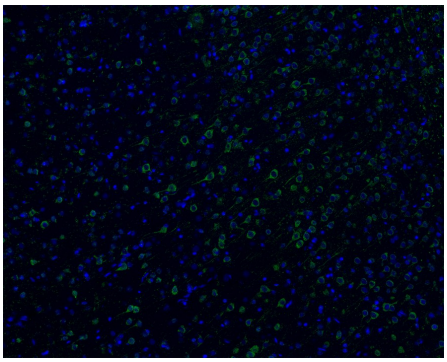

Fig7: Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling iNOS with Rabbit anti-iNOS antibody (ER1706-89).
The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ER1706-89, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

Background References

1. Hokari A et al. Cloning and functional expression of human inducible nitric oxide synthase (NOS) cDNA from a glioblastoma cell line A-172. J Biochem 116:575-581 (1994).
2. Guo F H et al. Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium in vivo. Proc Natl Acad Sci U.S.A. 92:7809-7813 (1995).