Product Data Sheet

iNOS Antibody

**Tested Species Reactivity**
- Human (Hu)
- Mouse (Ms)
- Rat (Rt)
- Rabbit (Rb)

**Published Species Reactivity**
- Human (Hu)
- Amphibian (Am)
- Mouse (Ms)
- Rat (Rt)

**Tested Applications**
- Western Blot (WB)
- Immunofluorescence (IF)
- Immunohistochemistry (IHC)

**Published Applications**
- Western Blot (WB)
- Immunocytochemistry (ICC)
- Immunohistochemistry (IHC)

**Details**
- **Catalog Number:** PA1-036
- **Size:** 200 µl
- **Class:** Polyclonal
- **Type:** Antibody
- **Clone:** Rabbit
- **Immunogen:** Synthetic peptide corresponding to residues D(17) L K E E K D I N N V K K T(31) of mouse iNOS.

**Form Information**
- **Form:** Liquid
- **Concentration:** 4mg/ml
- **Purification:** Ammonium sulfate precipitation
- **Storage Buffer:** PBS with 1mg/ml BSA
- **Preservative:** 0.05% sodium azide
- **Storage Conditions:** -20° C, Avoid Freeze/Thaw Cycles

**Product Specific Information**

PA1-036 detects inducible nitric oxide synthase (iNOS) from human, mouse and rat tissues and cells as well as recombinant human and mouse iNOS. This antibody does not detect other NOS isoforms.

PA1-036 has been successfully used in Western blot and immunofluorescence procedures. By Western blot, this antibody detects an ~135 kDa protein representing recombinant human iNOS and human iNOS from cytokine stimulated A549 cells. By Western blot PA1-036 also detects purified recombinant mouse iNOS, mouse iNOS from cytokine stimulated RAW 264.7 cells and cytokine stimulated rat fibroblast iNOS, though the signals are not as strong as those seen with the human samples.

The PA1-036 immunogen is a synthetic peptide corresponding to residues D(17) L K E E K D I N N V K K T(31) of mouse iNOS.

Nitric oxide (NO) is an inorganic, gaseous free radical that carries a variety of messages between cells. Vasorelaxation, neurotransmission and cytotoxicity can all be potentiated through cellular response to NO. NO production is mediated by members of the nitric oxide synthase (NOS) family. NOS catalyzes the oxidization of L-arginine to produce L-citrulline and NO. Two constitutive isoforms, brain or neuronal NOS (b or nNOS, type I) & endothelial cell NOS (eNOS, type III), and one inducible isoform (iNOS, type II), have been cloned. All NOS isoforms contain calmodulin, nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN) binding domains.

The PA1-036 recognizes iNOS in a variety of cell types including macrophages, hepatocytes, synoviocytes, and smooth muscle cells. Cytokines such as interferon-gamma (IFN), tumor necrosis factor (TNF), interleukin-1 and -2, and lipopolysaccharides (LPS) cause an increase in iNOS mRNA, protein, and activity levels. Protein kinase C-stimulating agents exhibit the same effect on iNOS activity. After cytokine induction, iNOS exhibits a delayed activity response which is then followed by a significant increase in NO production over a long period of time.

This product is for In Vitro experimental use only. Not for resale without express authorization.

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Immunofluorescence with anti-iNOS Polyclonal Antibody (PA1-036)

Immunofluorescent analysis of iNOS in A549 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with an iNOS polyclonal antibody (Product # PA1-036) at a dilution of 1:20 overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody (Product # 35503). iNOS staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.

Immunofluorescence with anti-iNOS Polyclonal Antibody (PA1-036)

Immunofluorescent analysis of iNOS in NIH-3T3 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with an iNOS polyclonal antibody (Product # PA1-036) at a dilution of 1:20 overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody (Product # 35503). iNOS staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.

Immunohistochemistry with anti-iNOS Polyclonal Antibody (PA1-036)

Immunohistochemistry was performed on normal deparaffinized human Heart tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a rabbit polyclonal antibody recognizing iNOS (Product #PA1-036) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP, followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

Immunohistochemistry with anti-iNOS Polyclonal Antibody (PA1-036)

Immunohistochemistry was performed on normal deparaffinized human Lung tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a rabbit polyclonal antibody recognizing iNOS (Product #PA1-036) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP, followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.
10 Western Blot References

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<thead>
<tr>
<th>Species / Dilution</th>
<th>Summary</th>
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</table>
PA1-036 was used in western blot to investigate the characteristics of nitric oxide synthase in vivo.

"Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase."
Author(s): Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH
Number of Citations: 1

PA1-036 was used in immunohistochemistry and western blot to investigate the functional properties of iNOS in the rat AO and PA treated with endotoxin.

"Differential inducible nitric oxide synthase expression in systemic and pulmonary vessels after endotoxin."
Author(s): Pulido EJ, Shames BD, Fullerton DA, Sheridan BC, Selzman CH, Gamboni-Robertson F, Bensard DD, McIntyre RC Jr
Number of Citations: 1

PA1-036 was used in western blot to investigate the effect of sivelestat on iNOS expression in liver cells stimulated with interleukin 1beta.

"Sivelestat suppresses iNOS gene expression in proinflammatory cytokine-stimulated hepatocytes."
Author(s): Araki Y, Matsumiya M, Matsuura T, Kaibori M, Okumura T, Nishizawa M, Kwon AH
Number of Citations: 0

1 Immunocytochemistry Reference

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<thead>
<tr>
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<tr>
<td>Ms / 0</td>
<td>PA1-036 was used in immunocytochemistry to study the role of Stat3 mammary gland regression</td>
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"Conditional deletion of Stat3 in mammary epithelium impairs the acute phase response and modulates immune cell numbers during post-lactational regression."
Author(s): Hughes K, Wickenden JA, Allen JE, Watson CJ
Number of Citations: 1

4 Immunohistochemistry References

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<tr>
<th>Species / Dilution</th>
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<td>Ms / Not Cited</td>
<td>PA1-036 was used in immunohistochemistry to study the role of the activated macrophages during the T cell-mediated chronic psoriasisform skin inflammation</td>
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"Activated macrophages are essential in a murine model for T cell-mediated chronic psoriasisform skin inflammation."
Number of Citations: 1

PA1-036 was used in immunohistochemistry to investigate the effect of TNFalpha on fatty liver formation induced by fructose.

"Role of tumor necrosis factor ? (TNF?) in the onset of fructose-induced nonalcoholic fatty liver disease in mice."
Author(s): Kanuri G, Spruss A, Wagnerberger S, Bischoff SC, Bergheim I
Number of Citations: 5

PA1-036 was used in immunohistochemistry and western blot to investigate the functional properties of iNOS in the rat AO and PA treated with endotoxin.

"Differential inducible nitric oxide synthase expression in systemic and pulmonary vessels after endotoxin."
Author(s): Pulido EJ, Shames BD, Fullerton DA, Sheridan BC, Selzman CH, Gamboni-Robertson F, Bensard DD, McIntyre RC Jr
Number of Citations: 1

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PA1-036 was used in immunohistochemistry to investigate the effect of lung inflammation blockade on brain cooling after heatstroke injury.


"Inhibition of acute lung inflammation and injury is a target of brain cooling after heatstroke injury."

Author(s): Hsi-Hsing Y, Ching-Ping C, Juei-Tang C, Lin MT

Number of Citations: 0