abcam

Product datasheet

Anti-iNOS antibody [EPR16635] ab178945

Recombinant RabMAb

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Overview

Product name	Anti-iNOS antibody [EPR16635]
Description	Rabbit monoclonal [EPR16635] to iNOS
Host species	Rabbit
Specificity	This antibody shows low affinity on human samples. Based on our preliminary data, this antibody is not suitable for THP1 (Human monocytic leukemia monocyte) cell lines in WB.
Tested applications	**Suitable for:** Indirect ELISA, WB, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: RAW 264.7 treated with 0.1 µg/mL LPS for 6 hours, HepG2 treated with 10 µg/mL LPS for 6 hours,whole cell lysates; Human fetal brain lysate; L6 treated with 50 ng/ml IL-1 beta, 20 ng/ml TNF-alpha and 100U/ml IFN-gamma for 24 h, whole cell lysate; ICC/IF: RAW 264.7 cells treated with LPS (0.1 µg/mL), for 6 hours. IP: RAW 264.7 whole cell lysate treated with 1µg/mL LPS for 24h.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16635
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab178945 in the following tested applications.

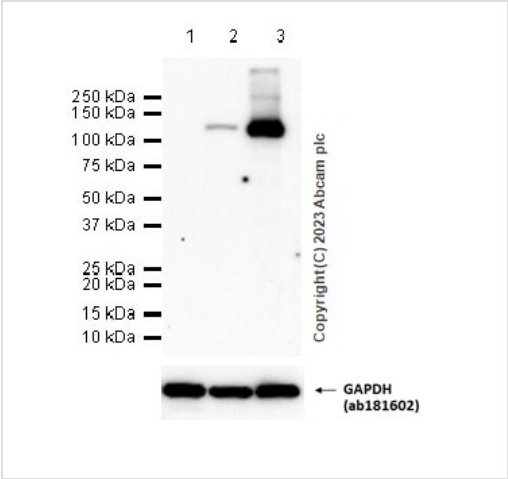
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Indirect ELISA		Use at an assay dependent concentration.
WB	★★★★☆ (1)	1/1000. Detects a band of approximately 131 kDa (predicted molecular weight: 131 kDa). To detect iNOS expression, macrophages need to be treated with LPS (0.1 - 1 ug/ml).
ICC/IF	★★★★★ (1)	1/500. To detect iNOS expression, macrophages need to be treated with LPS (0.1 - 1 ug/ml).
IP		1/100. To detect iNOS expression, macrophages need to be treated with LPS (0.1 - 1 ug/ml).

Target

Function	Produces nitric oxide (NO) which is a messenger molecule with diverse functions throughout the body. In macrophages, NO mediates tumoricidal and bactericidal actions. Also has nitrosylase activity and mediates cysteine S-nitrosylation of cytoplasmic target proteins such COX2.
Tissue specificity	Expressed in the liver, retina, bone cells and airway epithelial cells of the lung. Not expressed in the platelets.
Sequence similarities	Belongs to the NOS family. Contains 1 FAD-binding FR-type domain. Contains 1 flavodoxin-like domain.

Images



Western blot - Anti-iNOS antibody [EPR16635] (ab178945)

All lanes : Anti-iNOS antibody [EPR16635] (ab178945) at 1/1000 dilution

Lane 1 : Mouse hippocampus tissue lysate
Lane 2 : Mouse colon tissue lysate
Lane 3 : Mouse colon cancer tissue lysate

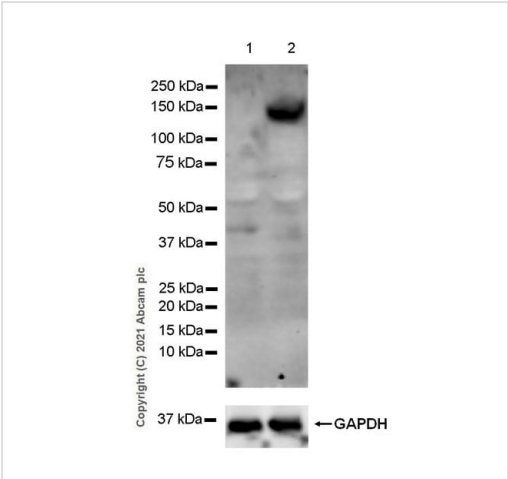
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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

Exposure time: 180 seconds

iNOS is not normally expressed in the brain, but can be induced in the brain after inflammatory, infectious, or other damages (PMID: 11138926, PMID: 16156895, PMID: 10322315).



Western blot - Anti-iNOS antibody [EPR16635] (ab178945)

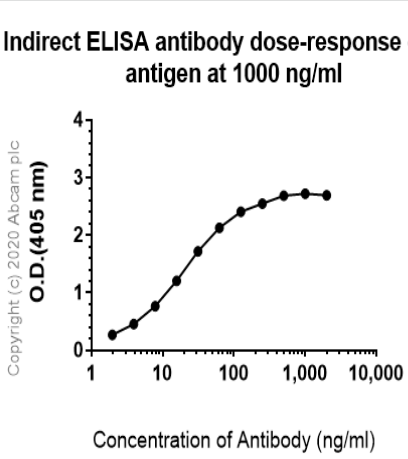
All lanes : Anti-iNOS antibody [EPR16635] (ab178945) at 1/1000 dilution

Lane 1 : Untreated L6 (rat skeletal muscle myoblast) whole cell lysate
Lane 2 : L6 treated with 50 ng/ml IL-1 beta, 20 ng/ml TNF-alpha and 100U/ml IFN-gamma for 24 h, whole cell lysate

Lysates/proteins at 20 µg per lane.

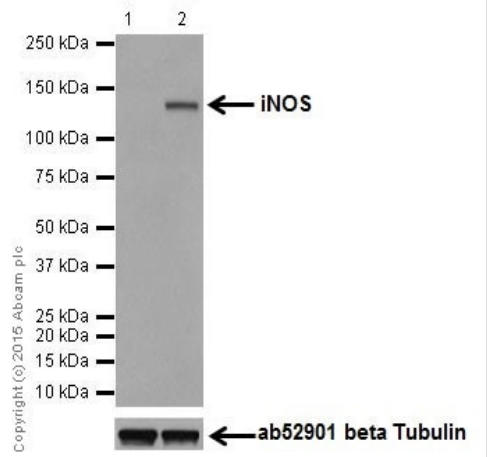
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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



Indirect ELISA - Anti-iNOS antibody [EPR16635] (ab178945)

ELISA analysis of House mouse iNOS recombinant protein at 1000 ng/mL with ab178945. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution was used as the secondary antibody.



Western blot - Anti-iNOS antibody [EPR16635] (ab178945)

All lanes : Anti-iNOS antibody [EPR16635] (ab178945) at 1/20000 dilution

Lane 1 : Untreated RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysate
Lane 2 : RAW 264.7 whole cell lysate treated with 0.1 µg/mL LPS for 6 hours

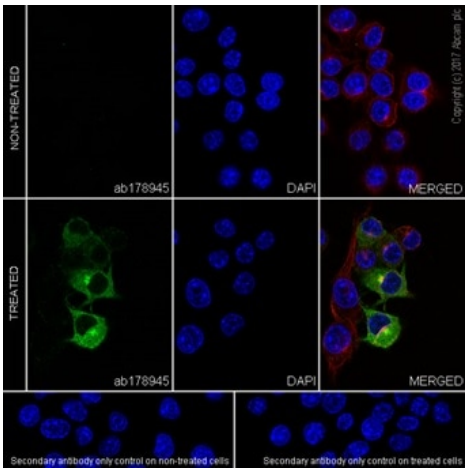
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

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

Exposure time: 30 seconds

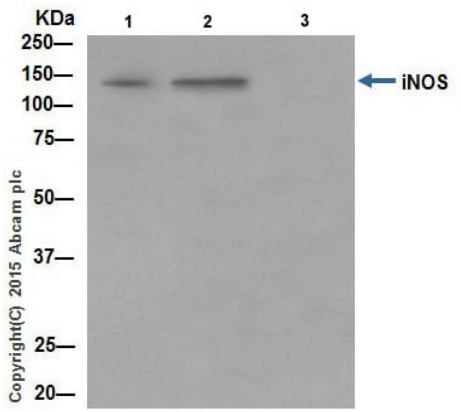
Blocking and dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-iNOS antibody [EPR16635] (ab178945)

Immunocytochemistry/Immunofluorescence analysis of RAW 264.7 non-treated and LPS treated (0.1 µg/mL) cells labelling iNOS with ab178945 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. The cells were co-stained with **ab195889**, Alexa Fluor® 594 conjugated anti-alpha tubulin (1/200). Nuclei counterstained with DAPI (blue).

Secondary antibody only controls performed on non-treated and treated cells.



Immunoprecipitation - Anti-iNOS antibody [EPR16635] (ab178945)

iNOS was immunoprecipitated from 1mg of RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysate treated with 1 µg/ml LPS for 24h with ab178945 at 1/100 dilution.

Lane 1: RAW 264.7 whole cell lysate treated with 1 µg/ml LPS for 24h,10ug (Input).

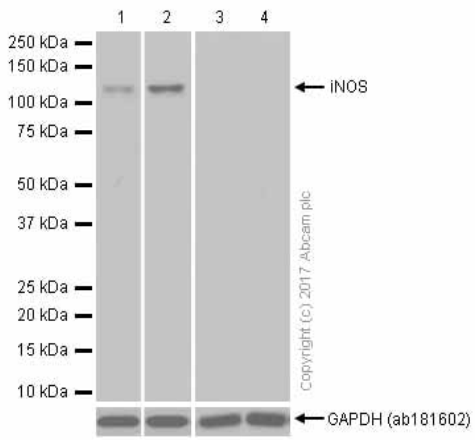
Lane 2: ab178945 IP in RAW 264.7 whole cell lysate treated with 1 µg/ml LPS for 24h.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab178945 in RAW 264.7 whole cell lysate treated with 1 µg/ml LPS for 24h.

Western blot was performed from the immunoprecipitate using ab178945 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1/1500.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.



Western blot - Anti-iNOS antibody [EPR16635] (ab178945)

All lanes : Anti-iNOS antibody [EPR16635] (ab178945) at 1/500 dilution

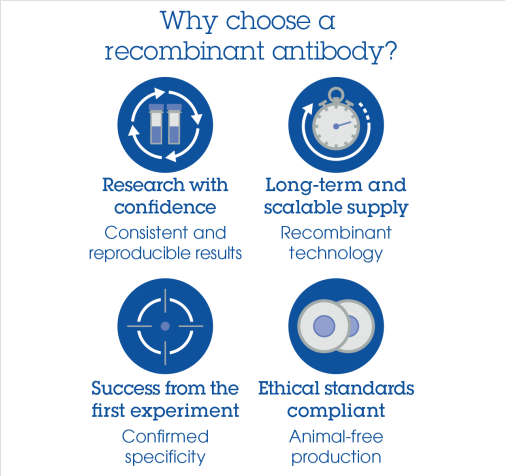
Lane 1 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates
Lane 2 : HepG2 (Human hepatocellular carcinoma epithelial cell) treated with 10ug/ml lipopolysaccharides for 6 hours whole cell lysates
Lane 3 : THP-1 (Human monocytic leukemia monocyte) whole cell lysates
Lane 4 : THP-1 (Human monocytic leukemia monocyte) treated with 100ng/ml lipopolysaccharides for 3 hours whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/200000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 131 kDa

Exposure time: 3 minutes



Anti-iNOS antibody [EPR16635] (ab178945)

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