abcam

Product datasheet

Anti-iNOS antibody [EPR16635] - BSA and Azide free ab213987

Recombinant RabMAb

14 References 9 Images

Overview

Product name	Anti-iNOS antibody [EPR16635] - BSA and Azide free
Description	Rabbit monoclonal [EPR16635] to iNOS - BSA and Azide free
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:** ICC/IF, Indirect ELISA, WB, 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	ab213987 is the carrier-free version of **ab178945**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16635
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab213987 in the following tested applications.

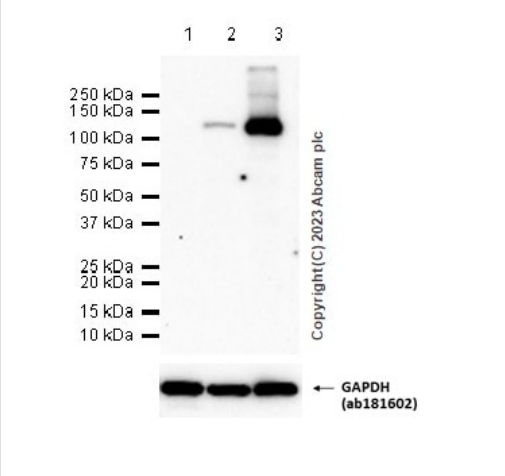
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/500.
Indirect ELISA		Use at an assay dependent concentration.
WB		1/1000. Detects a band of approximately 131 kDa (predicted molecular weight: 131 kDa).
IP		1/100.

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] - BSA and Azide free (ab213987)

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

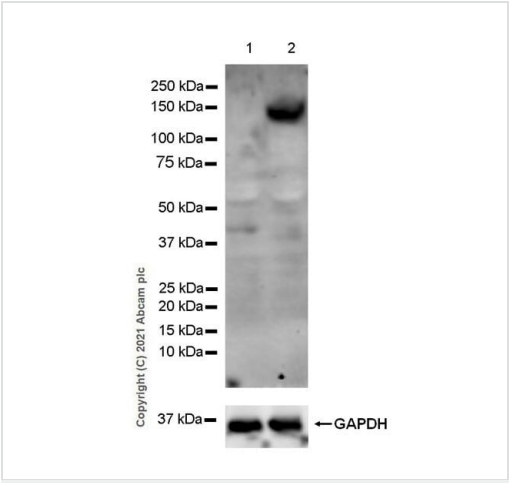
Predicted band size: 131 kDa
Observed band size: 131 kDa

Exposure time: 180 seconds

This data was developed using the same antibody clone in a different buffer formulation (**ab178945**).

Blocking and diluting buffer 5% NFDM/TBST.

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] - BSA and Azide free (ab213987)

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

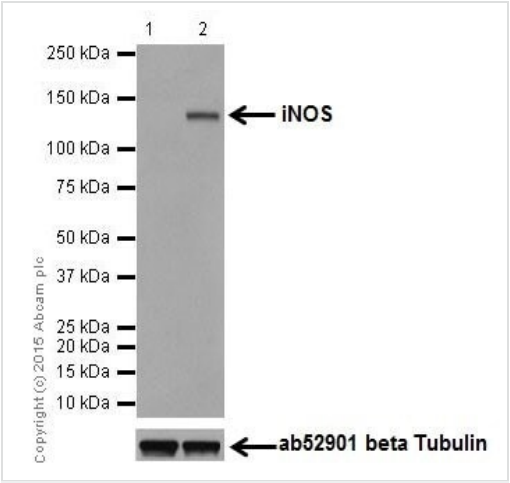
Lane 1 : Untreated L6 (rat skeletal muscle myoblast) whole cell lysate
Lane 2 : L6 treated with 50 ng/ml IL-1 beta, 20 ng/ml TNF-alpha and 100U/ml IFN-gamma for 24 h, whole cell lysate

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178945**).



Western blot - Anti-iNOS antibody [EPR16635] - BSA and Azide free (ab213987)

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.

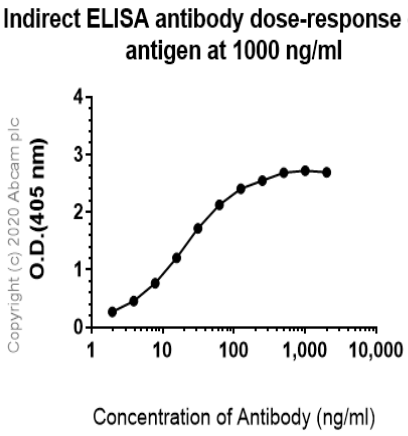
Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

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

Exposure time: 30 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

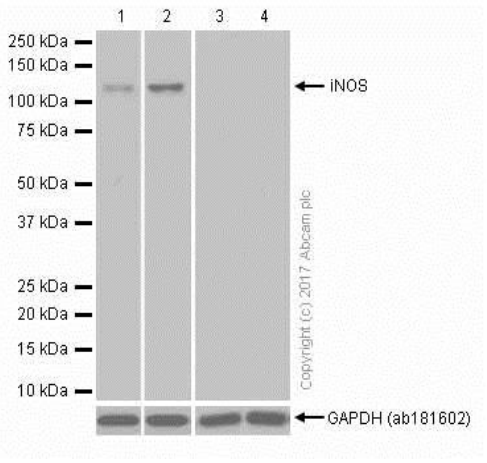
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178945**).



Indirect ELISA - Anti-iNOS antibody [EPR16635] - BSA and Azide free (ab213987)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178945**).



Western blot - Anti-iNOS antibody [EPR16635] - BSA and Azide free (ab213987)

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

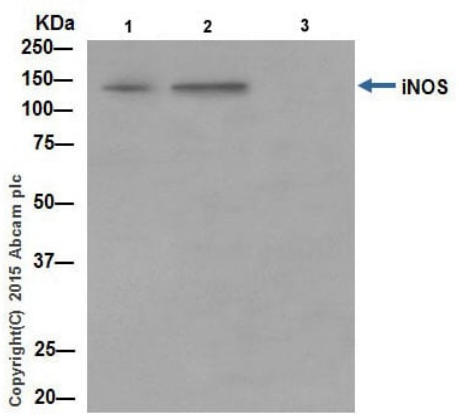
Lysates/proteins at 15 µg per lane.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178945**).



Immunoprecipitation - Anti-iNOS antibody [EPR16635] - BSA and Azide free (ab213987)

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.

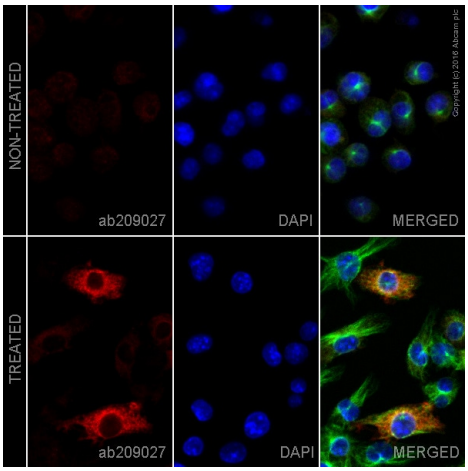
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab178945** in RAW 264.7 whole cell lysate treated with 1 µg/ml LPS for 24h.

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 dilution

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

Exposure time: 30 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178945**).



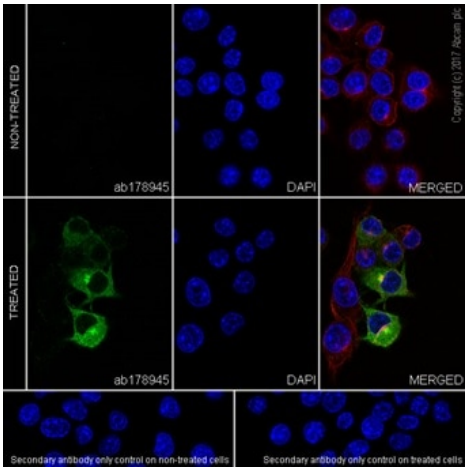
Immunocytochemistry/ Immunofluorescence - Anti-iNOS antibody [EPR16635] - BSA and Azide free (ab213987)

Clone EPR16635 (ab213987) has been successfully conjugated by Abcam. This image was generated using Anti-iNOS antibody [EPR16635] (Alexa Fluor® 647). Please refer to **ab209027** for protocol details.

Ab209027 staining iNOS in Raw264.7 cells. The lower panel shows cells treated with 1ug/ml Lipopolysaccharides (24 hr). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab209027**at 1/100 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in SW480 cells fixed with 4% formaldehyde (10 min).

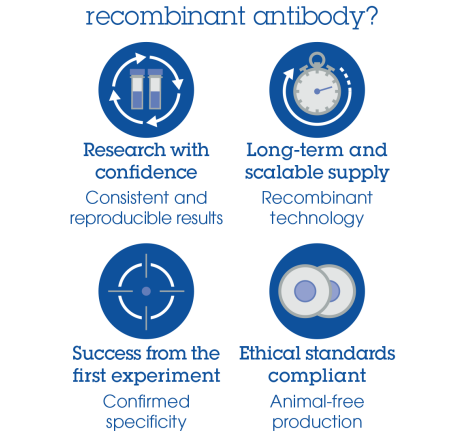


Immunocytochemistry/ Immunofluorescence - Anti-iNOS antibody [EPR16635] - BSA and Azide free (ab213987)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178945**).



Anti-iNOS antibody [EPR16635] - BSA and Azide free (ab213987)

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