**Product name**: Anti-iNOS antibody [EPR16635] ab178945

**Description**: Rabbit monoclonal [EPR16635] to iNOS

**Host species**: Rabbit

**Specificity**: This antibody shows low affinity on human samples. Based on our preliminary data, ab178945 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 buffer**: Preservative: 0.01% Sodium azide
Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
**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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indirect ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td>★★★★★ (1)</td>
<td>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).</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>★★★★★ (1)</td>
<td>1/500. To detect iNOS expression, macrophages need to be treated with LPS (0.1 - 1 ug/ml).</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>1/100. To detect iNOS expression, macrophages need to be treated with LPS (0.1 - 1 ug/ml).</td>
</tr>
</tbody>
</table>

**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**

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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

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.

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 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.

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.
**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 10µg/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
Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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