Anti-NOX2 / gp91phox Antibody [PSH10-59]

HA723224

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IHC-Fr, IP
Molecular Wt:	Predicted band size: 65 kDa
Clone number:	PSH10-59

Description: NADPH oxidase 2 (Nox2), also known as cytochrome b(558) subunit beta or Cytochrome b-245 heavy chain, is a protein that in humans is encoded by the NOX2 gene (also called CYBB gene). The protein is a superoxide generating enzyme which forms reactive oxygen species (ROS). The CYBB gene encode cytochrome b-245, beta chain. This protein is subunit of a group of proteins that forms an enzyme complex called NADPH oxidase, which plays an essential role in the immune system. Within this complex, the cytochrome b-245, beta chain has an alpha chain partner (produced from the CYBA gene). Both alpha and beta chains are required for either to function and the NADPH oxidase complex requires both chains in order to be functional. It has been proposed as a primary component of the microbicidal oxidase system of phagocytes.

Positive control: RAW264.7 cell lysate, Mouse spleen tissue lysate, RAW264.7, human liver tissue, mouse liver tissue, rat liver tissue.

Subcellular location: Cell membrane.

Database links: SwissProt: P04839 Human | Q61093 Mouse
Entrez Gene: 66021 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:500
IHC-P	1:1,000
IHC-Fr	1:500
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

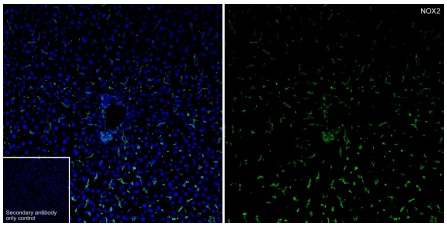

Fig1: Immunofluorescence analysis of frozen mouse liver tissue with Rabbit anti-NOX2 / gp91phox antibody (HA723224) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA723224, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

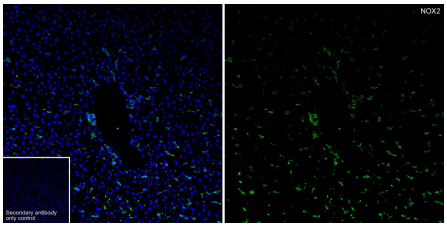

Fig2: Immunofluorescence analysis of frozen rat liver tissue with Rabbit anti-NOX2 / gp91phox antibody (HA723224) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA723224, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

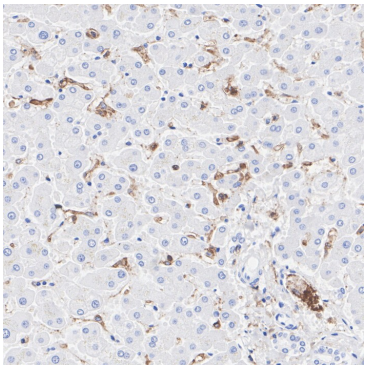

Fig3: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-NOX2 / gp91phox antibody (HA723224) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723224) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

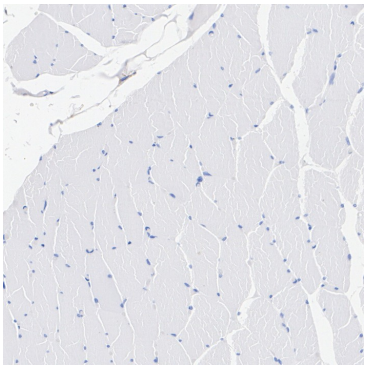

Fig4: Immunohistochemical analysis of paraffin-embedded human skeletal muscle tissue (negative) with Rabbit anti-NOX2 / gp91phox antibody (HA723224) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723224) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

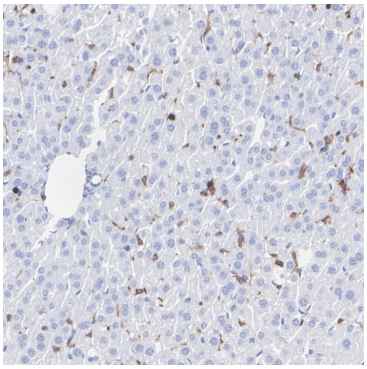

Fig5: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-NOX2 / gp91phox antibody (HA723224) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723224) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

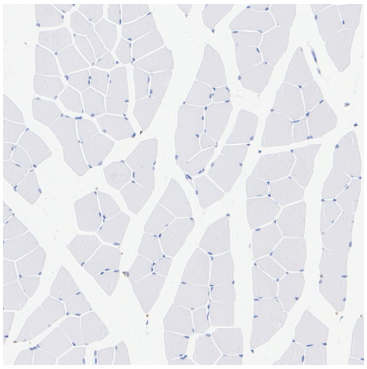

Fig6: Immunohistochemical analysis of paraffin-embedded mouse skeletal muscle tissue (negative) with Rabbit anti-NOX2 / gp91phox antibody (HA723224) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723224) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

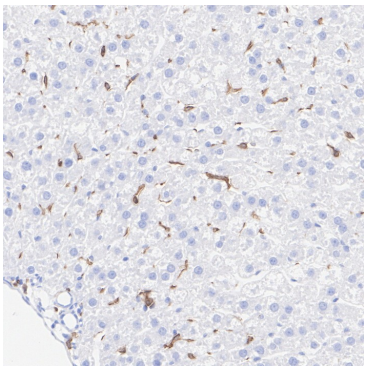

Fig7: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-NOX2 / gp91phox antibody (HA723224) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723224) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

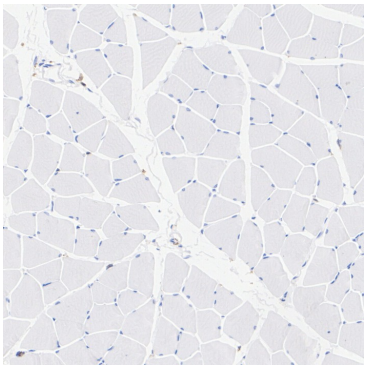

Fig8: Immunohistochemical analysis of paraffin-embedded rat skeletal muscle tissue (negative) with Rabbit anti-NOX2 / gp91phox antibody (HA723224) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723224) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

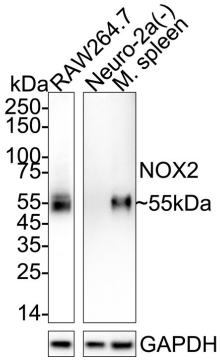

Fig9: Western blot analysis of NOX2 / gp91phox on different lysates with Rabbit anti-NOX2 / gp91phox antibody (HA723224) at 1/5,000 dilution.

Lane 1: RAW264.7 cell lysate (20 µg/Lane)
Lane 2: Neuro-2a cell lysate (negative) (20 µg/Lane)
Lane 3: Mouse spleen tissue lysate (20 µg/Lane)

Predicted band size: 65 kDa
Observed band size: 55 kDa

Exposure time: Lane 1: 16 seconds; Lane 2-3: 46 seconds; ECL: K1801;
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 (HA723224) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

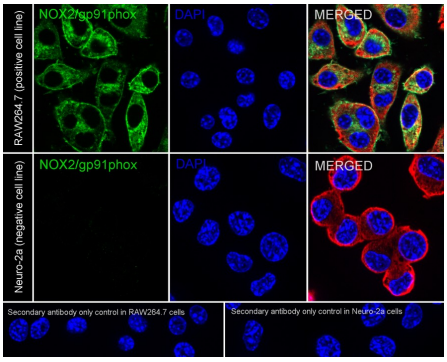

Fig10: Immunocytochemistry analysis of RAW264.7 (positive) and Neuro-2a (negative) labeling NOX2 / gp91phox with Rabbit anti-NOX2 / gp91phox antibody (HA723224) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-NOX2 / gp91phox antibody (HA723224) at 1/500 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 (HA601187, 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.

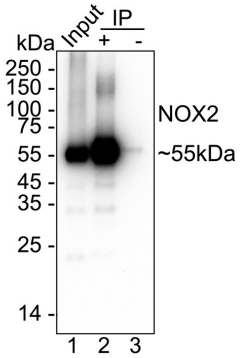

Fig11: NOX2 / gp91phox was immunoprecipitated from 0.2 mg RAW264.7 cell lysate with HA723224 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA723224 at 1/2,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: RAW264.7 cell lysate (input)
Lane 2: HA723224 IP in RAW264.7 cell lysate
Lane 3: Rabbit IgG instead of HA723224 in RAW264.7 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 1 3 seconds; ECL: K1801

Background References

1. Wang Y et al. NOX2 inhibition stabilizes vulnerable plaques by enhancing macrophage efferocytosis via MertK/PI3K/AKT pathway. Redox Biol. 2023 Aug
2. Hu C et al. Nox2 impairs VEGF-A-induced angiogenesis in placenta via mitochondrial ROS-STAT3 pathway. Redox Biol. 2021 Sep