Anti-Nrf2 Antibody

ER1706-41

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 68 kDa

Description: The NF-E2 DNA binding protein is composed of two subunits, p45 and MafK. It regulates expression of globin genes in developing erythroid cells through interaction with Maf recognition elements (Mares). A family of NF-E2- related proteins, which are collectively known as the Cap 'n' collar (CNC) family and include Nrf1 (also designated TCF11), Nrf2 and Nrf3, are bZIP transcription factors that heterodimerize with Maf proteins to bind Mare sequences. The Nrf proteins also bind the antioxidant response element (ARE) and are implicated in the regulation of detoxification enzymes and the oxidative stress response. They do so by heterodimerizing with Jun family members (c-Jun, Jun B and Jun D) to activate gene expression, specifically the detoxifying enzyme NQO1. Nrf2 is widely expressed and is thought to translocate to the nucleus after treatment with xenobiotics and antioxidants, which stimulate its release from its repressor protein, Keap1.

Immunogen: Synthetic peptide within N-terminal human Nrf2.

Positive control: Hela cell lysates, HCT116 cell lysates, HCT 116 cells treated with 10 μM MG-132 for 6 hours, MEF cells treated with 10 μM MG-132 for 6 hours, rat brain tissue, human lung tissue, mouse fallopian tubes tissue, human spleen tissue, human uterus tissue.

Subcellular location: Cytoplasm. Nucleus.

Database links: SwissProt: Q16236 Human | Q60795 Mouse | O54968 Rat

Recommended Dilutions:

WB	1:500
IF-Cell	1:100
IHC-P	1:50-1:200
FC	1:50-1:100

Storage Buffer: 1*TBS (pH7.4), 0.5% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Immunogen affinity purified.

Images

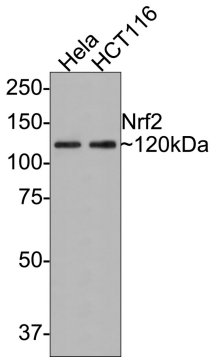

Fig1: Western blot analysis of Nrf2 on different lysates with Rabbit anti-Nrf2 antibody (ER1706-41) at 1/500 dilution.

Lane 1: Hela cell lysate
Lane 2: HCT116 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 68 kDa
Observed band size: 120 kDa

Exposure time: 2 minutes;

8% 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 (ER1706-41) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

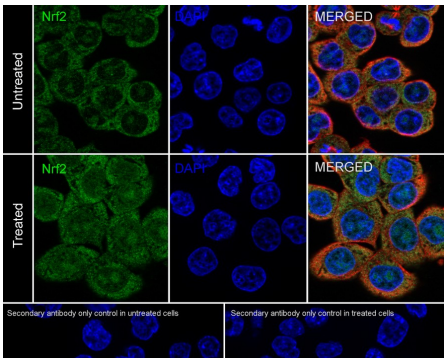

Fig2: Immunocytochemistry analysis of HCT 116 cells treated with or without 10 µM MG-132 for 6 hours labeling Nrf2 with Rabbit anti-Nrf2 antibody (ER1706-41) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Nrf2 antibody (ER1706-41) at 1/100 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 (M1305-2, 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.

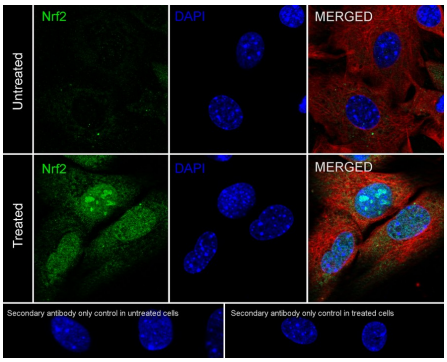

Fig3: Immunocytochemistry analysis of MEF cells treated with or without 10 μM MG-132 for 6 hours labeling Nrf2 with Rabbit anti-Nrf2 antibody (ER1706-41) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Nrf2 antibody (ER1706-41) at 1/100 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 (M1305-2, 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.

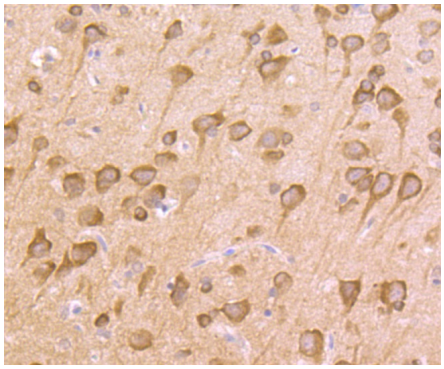

Fig4: Immunohistochemical analysis of paraffin-embedded rat brain tissue using anti-Nrf2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1706-41, 1/200) for 30 minutes 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.

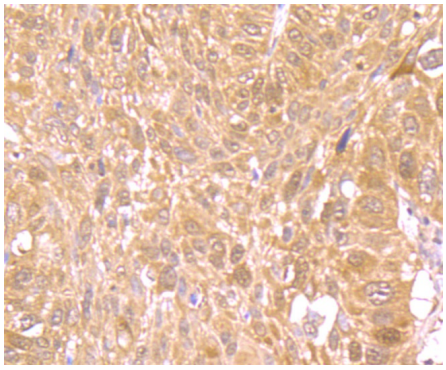

Fig5: Immunohistochemical analysis of paraffin-embedded human lung tissue using anti-Nrf2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1706-41, 1/100) for 30 minutes 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.

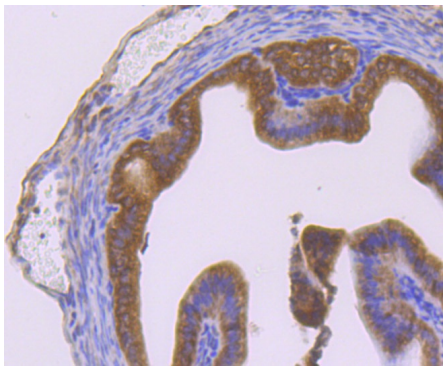

Fig6: Immunohistochemical analysis of paraffin-embedded mouse fallopian tubes tissue using anti-Nrf2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1706-41, 1/200) for 30 minutes 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.

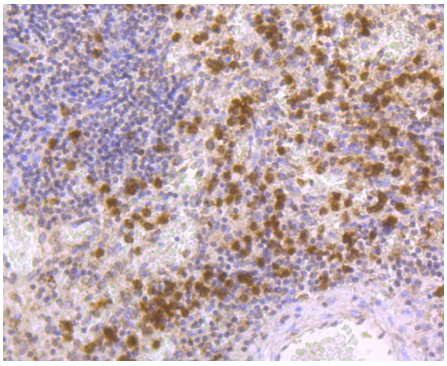

Fig7: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-Nrf2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1706-41, 1/200) for 30 minutes 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.

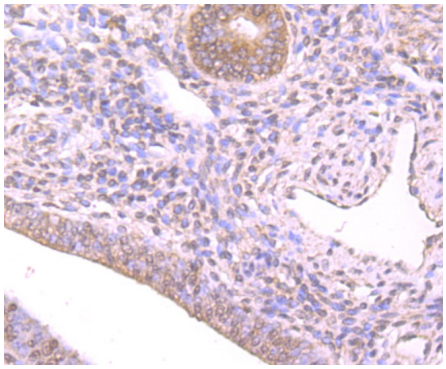

Fig8: Immunohistochemical analysis of paraffin-embedded human uterus tissue using anti-Nrf2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1706-41, 1/50) for 30 minutes 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.

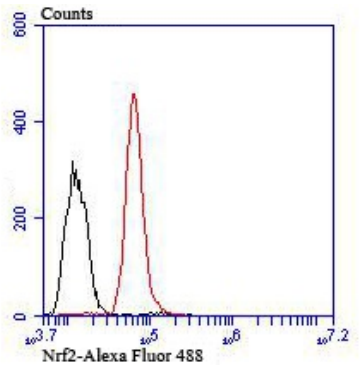

Fig9: Flow cytometric analysis of Nrf2 was done on HepG2 cells. The cells were fixed, permeabilized and stained with the primary antibody (ER1706-41, 1/100) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated goat anti-rabbit IgG Secondary antibody at 1/500 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Kerr F et al. Direct Keap1-Nrf2 disruption as a potential therapeutic target for Alzheimer's disease. PLoS Genet 13:e1006593 (2017).
2. Arai A et al. p62/SQSTM1 levels predicts radiotherapy resistance in hypopharyngeal carcinomas. Am J Cancer Res 7:881-891 (2017).