

Anti-NRF1 Antibody [JM89-63]

ET1705-86



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	JM89-63

Description: The NF-E2 DNA binding protein is composed of two subunits, p45 and MafK, and 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, JunB and JunD) 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 a repressor protein Keap1. Nrf3 is highly expressed in placenta, B cells and monocytes.

Immunogen: Synthetic peptide within Human NRF1 aa 333-382 / 503.

Positive control: HeLa cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, 293T, MCF7, human tonsil tissue, human thyroid tissue, mouse skeletal muscle tissue, mouse colon tissue, rat hippocampus tissue, rat brain tissue, 293T.

Subcellular location: Nucleus.

Database links: SwissProt: Q16656 Human | Q9WU00 Mouse | Q62792 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:200
FC	1:1,000
IP	Use at an assay dependent concentration.

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

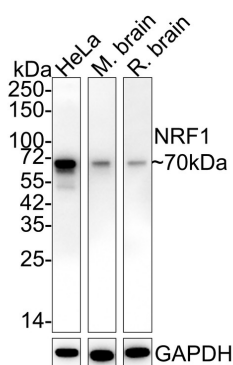


Fig1: Western blot analysis of NRF1 on different lysates with Rabbit anti-NRF1 antibody (ET1705-86) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)

Lane 2: Mouse brain tissue lysate (20 µg/Lane)

Lane 3: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 54 kDa

Observed band size: 70 kDa

Exposure time: 1 minute 2 seconds;

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 (ET1705-86) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

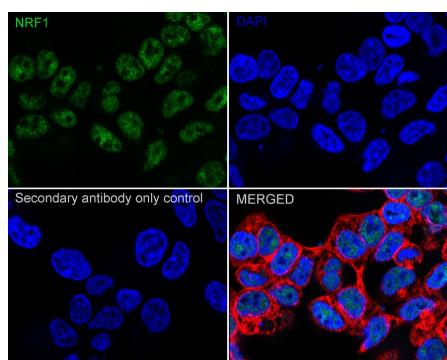


Fig2: Immunocytochemistry analysis of 293T cells labeling NRF1 with Rabbit anti-NRF1 antibody (ET1705-86) at 1/50 dilution.

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-NRF1 antibody (ET1705-86) at 1/50 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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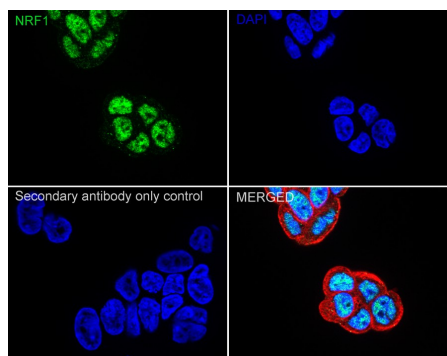
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Fig3: Immunocytochemistry analysis of MCF7 cells labeling NRF1 with Rabbit anti-NRF1 antibody (ET1705-86) at 1/100 dilution.



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-NRF1 antibody (ET1705-86) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

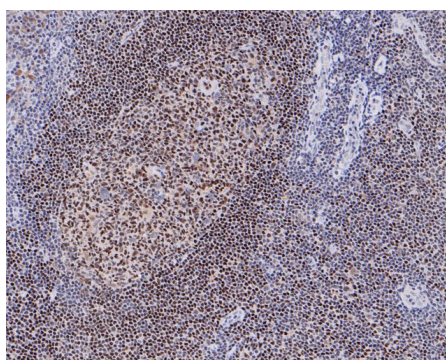


Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-NRF1 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1705-86, 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.

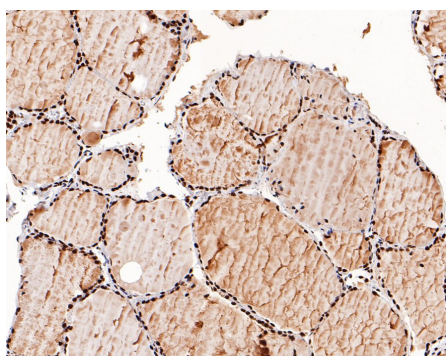


Fig5: Immunohistochemical analysis of paraffin-embedded human thyroid tissue with Rabbit anti-NRF1 antibody (ET1705-86) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1705-86) 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.

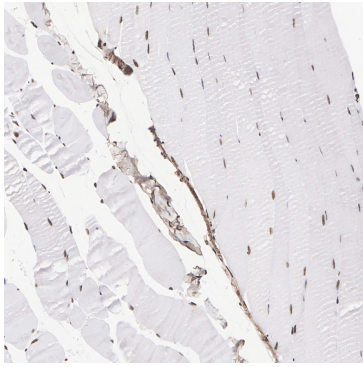


Fig6: Immunohistochemical analysis of paraffin-embedded mouse skeletal muscle tissue with Rabbit anti-NRF1 antibody (ET1705-86) at 1/200 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET1705-86) 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.

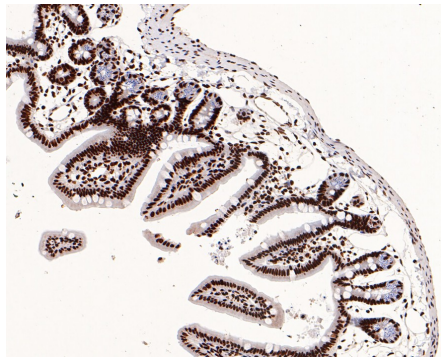


Fig7: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-NRF1 antibody (ET1705-86) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1705-86) 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.

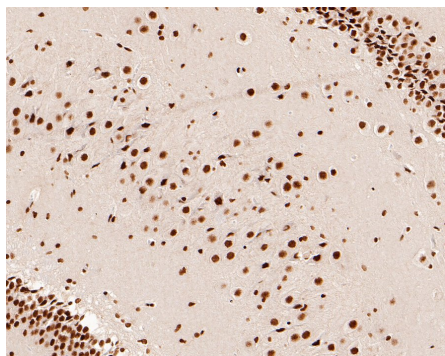


Fig8: Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Rabbit anti-NRF1 antibody (ET1705-86) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1705-86) 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.

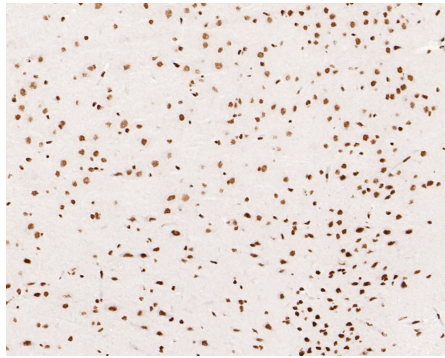


Fig9: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-NRF1 antibody (ET1705-86) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1705-86) 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.

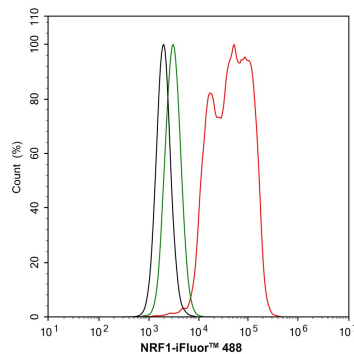


Fig10: Flow cytometric analysis of 293T cells labeling NRF1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1705-86, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are “FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE”.

Background References

1. Wang X et al. Hypoxia reduces testosterone synthesis in mouse Leydig cells by inhibiting NRF1-activated StAR expression. *Oncotarget* 8:16401-16413 (2017).
2. Kirienko NV et al. Mitophagy confers resistance to siderophore-mediated killing by *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 112:1821-6 (2015).

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