

Anti-Phospho-Nrf2 (S40) Antibody [SU0334]



ET1608-28

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: 68 kDa
Clone number:	SU0334

Description: Nuclear factor erythroid 2-related factor 2 (NRF2), also known as nuclear factor erythroid-derived 2-like 2, is a transcription factor that in humans is encoded by the NFE2L2 gene. NRF2 is a basic leucine zipper (bZIP) protein that may regulate the expression of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation, according to preliminary research.[6] In vitro, NRF2 binds to antioxidant response elements (AREs) in the promoter regions of genes encoding cytoprotective proteins. NRF2 induces the expression of heme oxygenase 1 in vitro leading to an increase in phase II enzymes. NRF2 also inhibits the NLRP3 inflammasome. NRF2 appears to participate in a complex regulatory network and performs a pleiotropic role in the regulation of metabolism, inflammation, autophagy, proteostasis, mitochondrial physiology, and immune responses.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser40 of Human Nrf2 aa 20-69 / 605.

Positive control: HepG2 cell lysate, Raji cell lysate, HepG2, Hela, A549, human breast carcinoma tissue, human tonsil tissue, human kidney tissue, K562.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt Q16236 Human

Recommended Dilutions:

WB	1:1,000-1:5,000
IF-Cell	1:50-1:250
IF-Tissue	1:50-1:200
IHC-P	1:50-1:1,000
FC	1:50-1:100
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% SodiumAzide.

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

Purity: Protein A affinity purified.

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Images

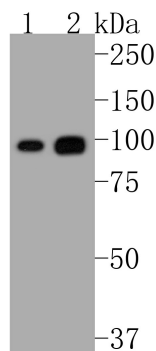


Fig1: Western blot analysis of Phospho-Nrf2 (S40) on different lysates with Rabbit anti-Phospho-Nrf2 (S40) antibody (ET1608-28) at 1/500 dilution.

Lane 1: HepG2 cell lysate

Lane 2: Raji cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 68 kDa

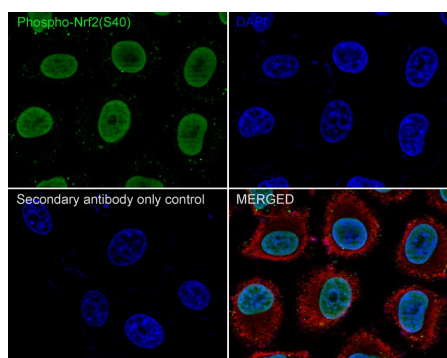
Observed band size: 100 kDa

Exposure time: 30 seconds;

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 (ET1608-28) 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 HepG2 cells labeling Phospho-Nrf2 (S40) with Rabbit anti-Phospho-Nrf2 (S40) antibody (ET1608-28) at 1/250 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-Phospho-Nrf2 (S40) antibody (ET1608-28) at 1/250 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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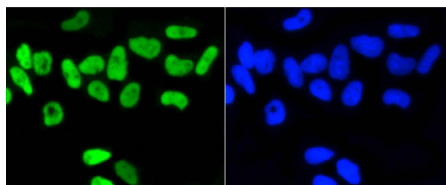


Fig3: ICC staining of Phospho-Nrf2 (S40) in HeLa cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1608-28, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

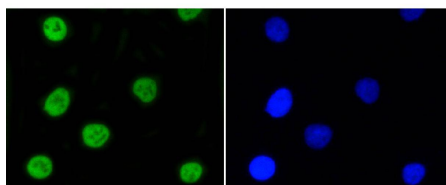


Fig4: ICC staining of Phospho-Nrf2 (S40) in A549 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1608-28, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

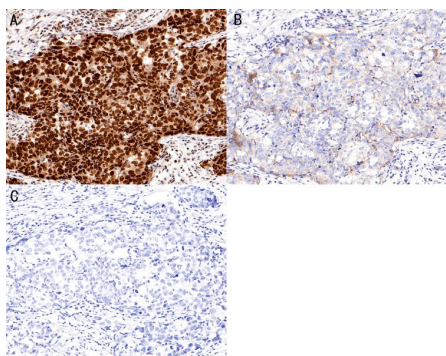


Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Phospho-Nrf2 (S40) antibody [SU0334] (ET1608-28). The section was pre-treated using heat mediated antigen retrieval with 10 mM sodium citrate buffer (pH 6.0). The section were untreated (A, C) and treated (B) with λ -PPase at 1/25 dilution at 30°C for 30 minutes after antigen retrieval. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1608-28, 1/200) for 30 minutes at room temperature. Goat anti-Rabbit IgG-HRP UltraPolymer antibody (HA1119) was used for 20 minutes at room temperature. DAB was used as the chromogen. The section were counterstained with hematoxylin and mounted with DPX. PBS was used instead of the primary antibody as the negative control, and is shown in the inset (C).

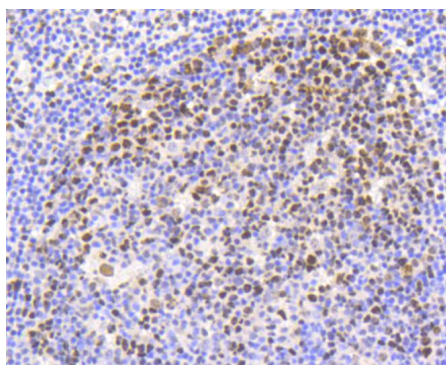


Fig6: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Phospho-Nrf2 (S40) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1608-28, 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.

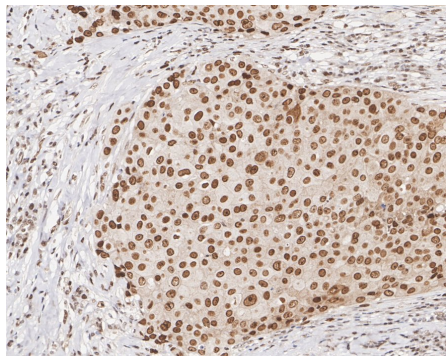


Fig7: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-Phospho-Nrf2 (S40) antibody (ET1608-28) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1608-28) 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.

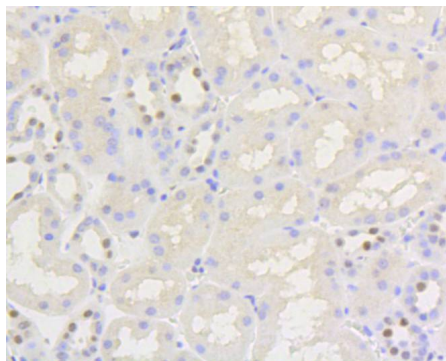


Fig8: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-Phospho-Nrf2 (S40) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1608-28, 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.

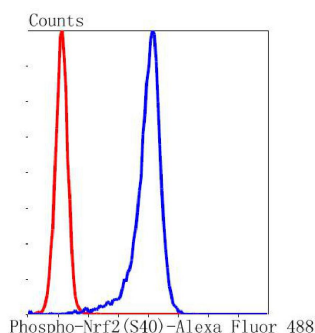


Fig9: Flow cytometric analysis of Phospho-Nrf2 (S40) was done on K562 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1608-28, 1/50) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes. Unlabelled sample was used as a control (cells without incubation with primary antibody; red).

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

Background References

1. Masuda Y et al. The effect of Nrf2 pathway activation on human pancreatic islet cells. PLoS One 10:e0131012 (2015).
2. Stachel I et al. Modulation of nuclear factor E2-related factor-2 (Nrf2) activation by the stress response gene immediate early response-3 (IER3) in colonic epithelial cells: a novel mechanism of cellular adaption to inflammatory stress. J Biol Chem 289:1917-29 (2014).

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