

p62/KEAP1/NRF2 Pathway Antibody Sampler Kit

HAK21140



Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Heme Oxygenase 1 (HO-1) [HA721854]	20μl	WB, IF-Cell, IHC-P	H, M	33 kDa
NQO1 [ET1702-50]	20μl	WB, IP, FC, IHC-P	H, M	31 kDa
SQSTM1 / p62 [HA721171]	20μl	WB, IHC-P, IF-Cell, FC, IF-Tissue, IHC-Fr	H, M, R	48 kDa
Phospho-SQSTM1 / p62 (S349) [HA723050]	20μl	WB, IF-Cell, IHC-P	H, M	48 kDa
MAP1LC3A [ET1609-26]	20μl	WB, IF-Cell, FC, IP	H, M, R	14 kDa
Keap1 [HA721525]	20μl	WB, IHC-P, FC	H, M, R	70 kDa
Nrf2 [HA723302]	20μl	WB, IF-Cell, ChIP	H, M, R	68 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [ha1001]	100μl	WB, ELISA, IHC-P	Rab	

Description: The p62/KEAP1/NRF2 Pathway Antibody Sampler Kit provides an economical means of detecting the non-canonical mechanism of NRF2 activation involving autophagy. The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

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

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

Background The cap 'n' collar (CNC), leucine zipper (bZIP) transcription factor NRF2 (also called nuclear factor erythroid 2-related factor 2 (NFE2L2)) is the master regulator of the cellular antioxidant response, regulating the expression of over 200 genes that contain antioxidant response elements (AREs) in their regulatory regions by heterodimerizing with small MAF proteins. Under normal conditions, KEAP1 binds to and targets NRF2 for ubiquitination-dependent proteasomal degradation. Upon oxidative stress, KEAP1 is modified on some sensor cysteines, affecting its conformation and thus interfering its binding to NRF2, allowing newly synthesized NRF2 to accumulate and translocate to the nucleus to activate its target genes, including HO-1 (heme oxygenase 1) and NQO1 (NAD(P)H:quinone oxidoreductase 1). Another mode of NRF2 regulation involves the autophagy adapter protein p62 (or sequestosome 1 [SQSTM1]) in a KEAP1-dependent but cysteine-independent manner, the so called non-canonical pathway. This process may also require autophagy protein LC3.

Database links: UniProt ID: P09601, P14901, P15559, Q64669, P05982, Q13501, Q64337, O08623, Q13501, Q64337, Q9H492, Q91VR7, Q6XVN8, Q14145, Q9Z2X8, P57790, Q16236, Q60795, O54968

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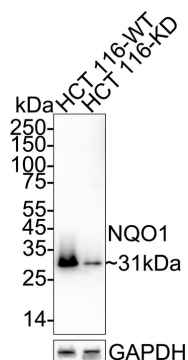
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Images

Fig1: Western blot analysis of NQO1 on different lysates with Rabbit anti-NQO1 antibody (ET1702-50) at 1/2,000 dilution.

Lane 1: HCT 116-si NT cell lysate
Lane 2: HCT 116-si NQO1 cell lysate



Lysates/proteins at 10 µg/Lane.

Predicted band size: 31 kDa

Observed band size: 31 kDa

Exposure time: 16 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 (ET1702-50) 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.

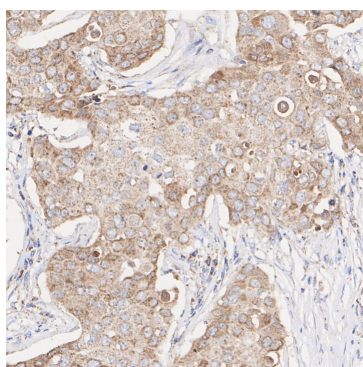


Fig2: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-NQO1 antibody (ET1702-50) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET1702-50) 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.

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Fig3: Application: IHC-Fr

Species: Mouse

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven.

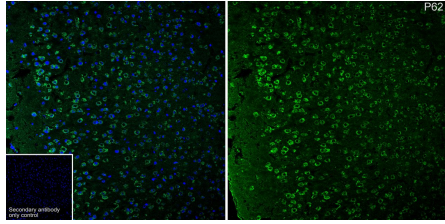


Fig4: Application: IF-tissue

Species: Mouse

Site: Liver

Sample: Paraffin-embedded section

Antibody concentration: 1/500

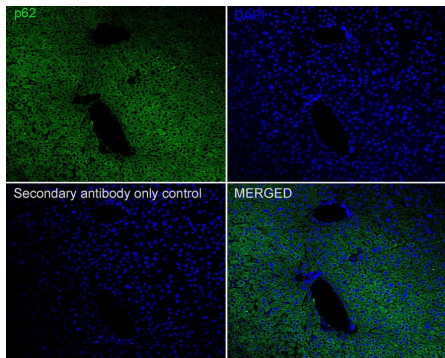


Fig5: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/1,000 dilution.

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 (HA721171) 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.

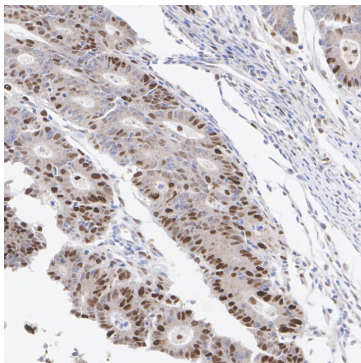
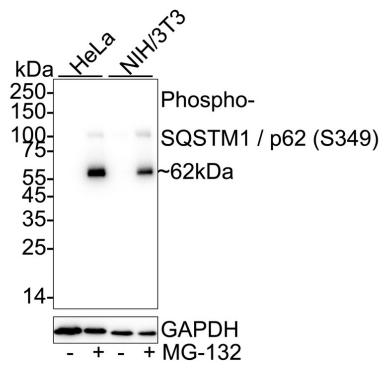


Fig6: Western blot analysis of Phospho-SQSTM1 / p62 (S349) on different lysates with Rabbit anti-Phospho-SQSTM1 / p62 (S349) antibody (HA723050) at 1/2,000 dilution.



Lane 1: HeLa cell lysate

Lane 2: HeLa treated with 2 μ M MG-132 for 18 hours cell lysate

Lane 3: NIH/3T3 cell lysate

Lane 4: NIH/3T3 treated with 10 μ M MG-132 for 8 hours cell lysate

Lysates/proteins at 20 μ g/Lane.

Predicted band size: 48 kDa

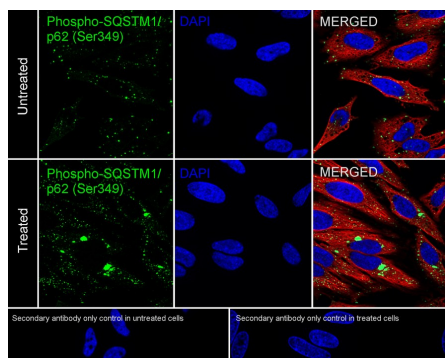
Observed band size: 62 kDa

Exposure time: 11 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (HA723050) at 1/2,000 dilution was used in 5% NFDN/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.

Fig7: Immunocytochemistry analysis of HeLa cells treated with 2 μ M MG-132 for 18 hours labeling Phospho-SQSTM1 / p62 (S349) with Rabbit anti-Phospho-SQSTM1 / p62 (S349) antibody (HA723050) at 1/5,000 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-Phospho-SQSTM1 / p62 (S349) antibody (HA723050) at 1/5,000 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 (HA601187, 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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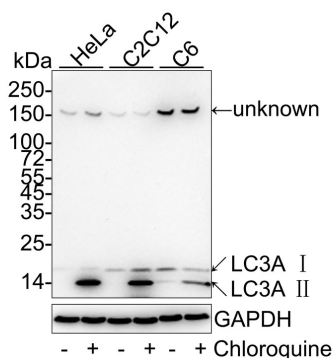
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Fig8: Western blot analysis of MAP1LC3A on different lysates with Rabbit anti-MAP1LC3A antibody (ET1609-26) at 1/1,000 dilution.



Lane 1: HeLa cell lysate

Lane 2: HeLa treated with 50 μ M Chloroquine for 18 hours cell lysate

Lane 3: C2C12 cell lysate

Lane 4: C2C12 treated with 50 μ M Chloroquine for 18 hours cell lysate

Lane 5: C6 cell lysate

Lane 6: C6 treated with 50 μ M Chloroquine for 18 hours cell lysate

Lysates/proteins at 20 μ g/Lane.

Predicted band size: 14 kDa

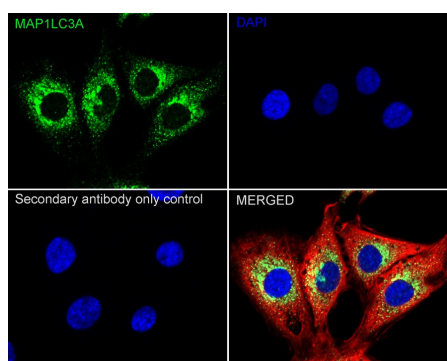
Observed band size: 14/16 kDa

Exposure time: 3 minutes; 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 (ET1609-26) at 1/1,000 dilution was used in 5% NFDM/TBST at 4 $^{\circ}$ C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig9: Immunocytochemistry analysis of C6 cells labeling MAP1LC3A with Rabbit anti-MAP1LC3A antibody (ET1609-26) 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-MAP1LC3A antibody (ET1609-26) at 1/100 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluorTM 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 $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluorTM 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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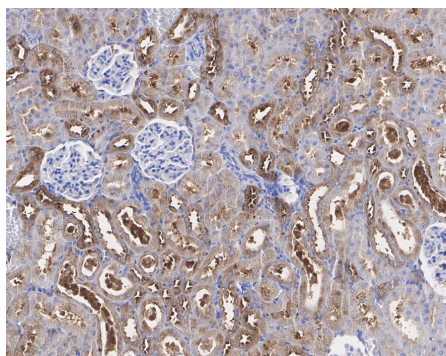
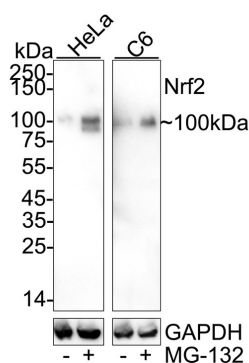


Fig10: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Keap1 antibody (HA721525) at 1/1,000 dilution.

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 (HA721525) 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.

Fig11: Western blot analysis of Nrf2 on different lysates with Rabbit anti-Nrf2 antibody (HA723302) at 1/2,000 dilution.



Lane 1: HeLa cell lysate

Lane 2: HeLa treated with 2 μ M MG-132 for 18 hours cell lysate

Lane 3: C6 cell lysate

Lane 4: C6 treated with 25 μ M MG-132 for 4 hours cell lysate

Lysates/proteins at 20 μ g/Lane.

Predicted band size: 68 kDa

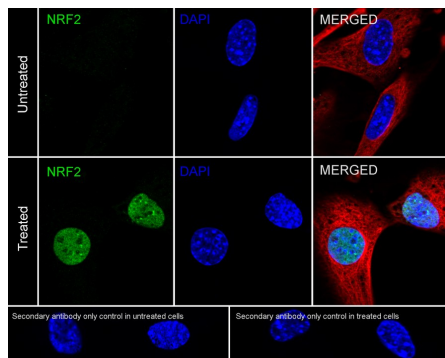
Observed band size: 100 kDa

Exposure time: 2 minutes; 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 (HA723302) at 1/2,000 dilution was used in 5% NFDM/TBST at 4 $^{\circ}$ C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig12: Immunocytochemistry analysis of MEF cells untreated / treated with 2 μ M MG-132 for 18 hours labeling Nrf2 with Rabbit anti-Nrf2 antibody (HA723302) 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-Nrf2 antibody (HA723302) 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 (HA601187, 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.

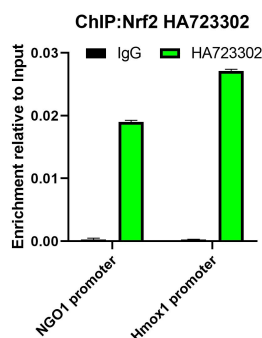


Fig13: Chromatin immunoprecipitations were performed with cross-linked chromatin from MEF cells treated with 10 μ M MG-132 for 8 hours with Nrf2 (HA723302) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

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

Background References

1. Yamamoto M, Kensler TW, Motohashi H. The KEAP1-NRF2 System: a Thiol-Based Sensor-Effector Apparatus for Maintaining Redox Homeostasis. *Physiol Rev.* 2018 Jul 1;98(3):1169-1203.
2. Bellezza I, Giambanco I, Minelli A, Donato R. Nrf2-Keap1 signaling in oxidative and reductive stress. *Biochim Biophys Acta Mol Cell Res.* 2018 May;1865(5):721-733.
3. Zhu M, Fahl WE. Functional characterization of transcription regulators that interact with the electrophile response element. *Biochem Biophys Res Commun.* 2001 Nov 23;289(1):212-9.

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