abcam

ab180845

Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free

Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845) is a rabbit recombinant monoclonal antibody provided in a PBS only buffer for easy conjugation detecting Nrf2 in **Western Blot, Flow Cytometry (Intra), ICC/IF**. Suitable for **Human**.

- Clone EP1808Y is the most cited clone to Nrf2
- KO validated for confirmed specificity
- BSA, sodium azide, and glycerol-free for easy conjugation
- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP1808Y
Purification technique	Affinity purification Protein A
Specificity	**The expression of Nrf2 is stimulated by oxidative stress, electrophiles and chemical activators (PMID: 25761198, PMID: 27638861 and PMID: 28587109). Nrf2 antibody (ab62352) detects no signal in most untreated samples in WB. Stimuli treated samples are recommended. We do not recommend using this product in western blot with tissue lysates, however some customers have used this antibody successfully using concentrated samples (see submitted abreviews).**
Concentration	1.039 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

IP

Not recommended

Species	Human
Dilution info	-
Notes	-

ChIP

Not recommended

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human
Dilution info	-
Notes	-

Target data

See full target information NFE2L2

Function

Transcription factor that plays a key role in the response to oxidative stress: binds to antioxidant response (ARE) elements present in the promoter region of many cytoprotective genes, such as phase 2 detoxifying enzymes, and promotes their expression, thereby neutralizing reactive electrophiles (PubMed:11035812, PubMed:19489739, PubMed:29018201, PubMed:31398338). In normal conditions, ubiquitinated and degraded in the cytoplasm by the BCR(KEAP1) complex (PubMed:11035812, PubMed:15601839, PubMed:29018201). In response to oxidative stress, electrophile metabolites inhibit activity of the BCR(KEAP1) complex, promoting nuclear accumulation of NFE2L2/NRF2, heterodimerization with one of the small Maf proteins and binding to ARE elements of cytoprotective target genes (PubMed:19489739, PubMed:29590092). The NFE2L2/NRF2 pathway is also activated in response to selective autophagy: autophagy promotes interaction between KEAP1 and SQSTM1/p62 and subsequent inactivation of the BCR(KEAP1) complex, leading to NFE2L2/NRF2 nuclear accumulation and expression of cytoprotective genes (PubMed:20452972). The NFE2L2/NRF2 pathway is also activated during the unfolded protein response (UPR), contributing to redox homeostasis and cell survival following endoplasmic reticulum stress (By similarity). May also be involved in the transcriptional activation of genes of the beta-globin cluster by mediating enhancer activity of hypersensitive site 2 of the beta-globin locus control region (PubMed:7937919). Also plays an important role in the regulation of the innate immune response and antiviral cytosolic DNA sensing. It is a critical regulator of the innate immune response and survival during sepsis by maintaining redox homeostasis and restraint of the dysregulation of pro-inflammatory signaling pathways like MyD88-dependent and -independent and TNF signaling (By similarity). Suppresses macrophage inflammatory response by blocking pro-inflammatory cytokine transcription and the induction of IL6 (By similarity). Binds to the proximity of pro-inflammatory genes in macrophages and inhibits RNA Pol II recruitment. The inhibition is independent of the NRF2-binding motif and reactive oxygen species level (By similarity). Represses antiviral cytosolic

DNA sensing by suppressing the expression of the adapter protein STING1 and decreasing responsiveness to STING1 agonists while increasing susceptibility to infection with DNA viruses (PubMed:30158636). Once activated, limits the release of pro-inflammatory cytokines in response to human coronavirus SARS-CoV-2 infection and to virus-derived ligands through a mechanism that involves inhibition of IRF3 dimerization. Also inhibits both SARS-CoV-2 replication, as well as the replication of several other pathogenic viruses including Herpes Simplex Virus-1 and-2, Vaccinia virus, and Zika virus through a type I interferon (IFN)-independent mechanism (PubMed:33009401).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

What is this antibody validated in?
Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunocytochemistry/immunofluorescence (ICC/IF) in Human samples.

What is the molecular weight of Nrf2?
Anti-Nrf2 [EP1808Y] - BSA and Azide free (ab180845) specifically detects a band for Nrf2 (UniProt: Q16236) at a molecular weight of 68kDa.

Trusted by the scientific community
Anti-Nrf2 [EP1808Y] - BSA and Azide free (ab180845) was first used in a scientific publication in 2016 and has been cited over 10 times in peer-reviewed journals.

Specificity confirmed
The specificity of Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845) has been confirmed by Western blot testing in NFE2L2 Knockout A549 cell line, ab285359.

Other related products
We have a range of other formats of antibody clone [EP1808Y] also available for your convenience: ab62352, Carrier free - ab180845, Alexa Fluor® 488 - ab194984, Alexa Fluor® 647 - ab194985, Alexa Fluor® 594 - ab206890, PE - ab223926, APC - ab223927

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For

details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Nrf2 also known as nuclear factor erythroid 2-related factor 2 is a transcription factor with a molecular weight of approximately 66 kDa. It plays a mechanical role in regulating the expression of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation. Nrf2 is ubiquitously expressed in various tissues including the liver lungs and skin. Nrf2 activation occurs when it translocates from the cytoplasm to the nucleus to bind the antioxidant response element (ARE) in the DNA starting transcription of target genes.
Biological function summary	Nrf2 acts as an important regulator of the cellular antioxidant response. It works in conjunction with its partner protein Keap1 forming a complex that controls its stability and degradation. Under normal conditions Keap1 keeps Nrf2 in the cytoplasm where it is targeted for ubiquitination and degradation. Once activated by oxidative stress or electrophiles Nrf2 dissociates from Keap1 thereby avoiding degradation and relocates to the nucleus to activate the transcription of ARE-dependent genes. This activity boosts the cellular response to oxidative stress by inducing genes involved in detoxification and cellular defense.
Pathways	Nrf2 plays a significant role in the oxidative stress response and detoxification pathways. Nrf2 activation is linked closely to the PI3K/Akt signaling pathway which influences cell survival growth and metabolism. This pathway also interacts with other important proteins like GSK-3B which can modulate Nrf2 activity and stability. Through these pathways Nrf2 orchestrates a defense mechanism against reactive oxygen species (ROS) by boosting the expression of antioxidant enzymes and detoxifying proteins.

Associated diseases and disorders	Nrf2 has been associated with conditions like cancer and neurodegenerative diseases. In cancer aberrant Nrf2 activation may lead to enhanced tumor survival by increasing expression of cytoprotective genes which makes cancer cells resistant to chemotherapy. Nrf2 also interacts with proteins such as p53 which play roles in tumor suppression and cellular stress responses. In neurodegenerative disorders reduced Nrf2 activity can contribute to oxidative stress leading to neuron damage and disease progression with proteins like amyloid-beta also being linked with oxidative processes affected by Nrf2 functionality.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

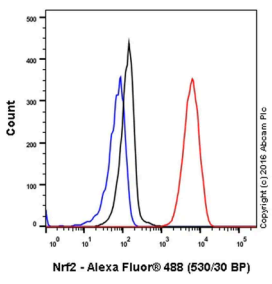
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

11 product images

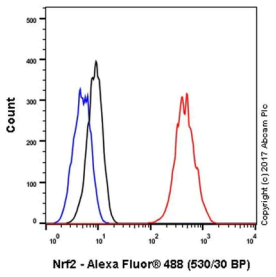


Flow Cytometry (Intracellular) - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

Nrf2 Flow Cytometry (Intracellular) staining using rabbit Anti-Nrf2 antibody

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labelling Nrf2 with ab62352 at 1/40 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. An Alexa Fluor®488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

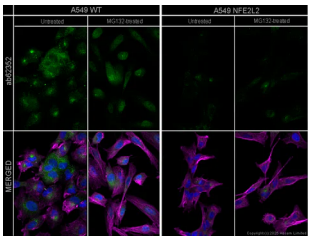
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab62352).



Flow Cytometry (Intracellular) - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

Nrf2 Flow Cytometry (Intracellular) staining using rabbit Anti-Nrf2 antibody

Intracellular Flow Cytometry analysis of HeLa cells labelling Nrf2 with purified ab62352 at a dilution of 1/60 (red). Cells were fixed with 4% paraformaldehyde. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG (ab172730). Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab62352).

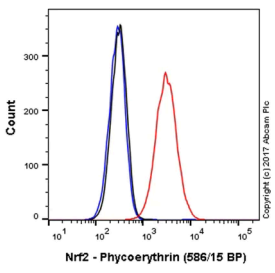


Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

Nrf2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Nrf2 antibody

This data was developed using ab62352, the same antibody clone in a different buffer formulation. ab62352 staining Nrf2 in untreated wild type A549 cells (first panel), treated wild type A549 cells (second panel), untreated NFE2L2 knockout A549 cells (third panel) and treated NFE2L2 knockout A549 cells (fourth panel). Cells were treated with 2µM of MG-132 for 18 hours (ab141003). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab62352 at 0.2 µg/ml concentration and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2µg/ml (shown in magenta). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Flow Cytometry (Intracellular) - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

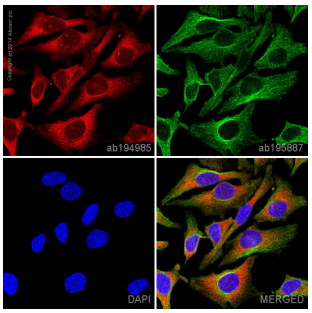
Nrf2 Flow Cytometry (Intracellular) staining using rabbit Anti-Nrf2 antibody

Clone EP1808Y (ab180845) has been successfully conjugated by Abcam. This image was generated using Anti-Nrf2 antibody [EP1808Y] (PE). Please refer to ab223926 for protocol details.
Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with ab223926 (red line). The cells were fixed with 4% formaldehyde (10 minutes) and then permeabilized with 0.1% PBS-Triton X-100 for 15 minutes. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab223926, 1/5000 dilution) for 30 minutes at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Phycoerythrin (ab209478) used at the same concentration and conditions as the primary antibody. Unlabeled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter.

This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 minutes)/permeabilized with 0.1% PBS-Triton X-100 for 15 minutes used under the same conditions.



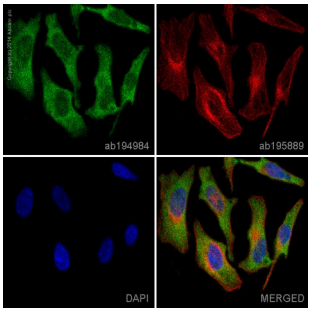
Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

Nrf2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Nrf2 antibody

Clone EP1808Y (ab180845) has been successfully conjugated by Abcam. This image was generated using Anti-Nrf2 antibody [EP1808Y] (Alexa Fluor® 647). Please refer to ab194985 for protocol details.
ab194985 staining Nrf2 in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab194985 at a working dilution 1/100 (shown in red) and ab195887, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 488, shown in green) at 2µg/ml overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

This product gave a positive signal in 4% formaldehyde (10 min) fixed HeLa cells under the same testing conditions.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

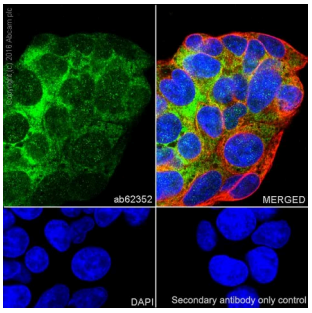


Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

Nrf2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Nrf2 antibody

Clone EP1808Y (ab180845) has been successfully conjugated by Abcam. This image was generated using Anti-Nrf2 antibody [EP1808Y] (Alexa Fluor® 488). Please refer to ab194984 for protocol details.
ab194984 staining Nrf2 in HeLa cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab194984 at a working dilution of 1/100 (shown in green) and ab195889, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 594, shown in red) at 2µg/ml overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



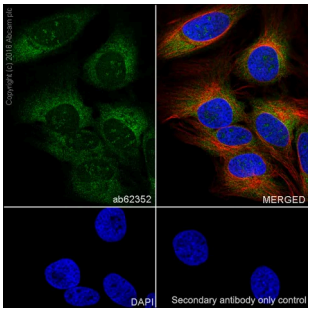
Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

Nrf2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Nrf2 antibody

Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling Nrf2 with purified ab62352 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. The cells were co-stained with ab195889, an Alexa Fluor® 594-conjugated mouse anti-alpha tubulin antibody (1/200). Nuclei counterstained with DAPI (blue).

Secondary antibody only control: PBS was used instead of the primary antibody as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab62352).

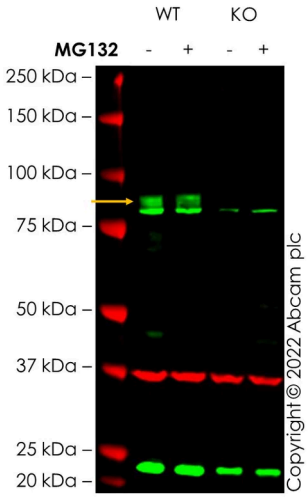


Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

Nrf2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Nrf2 antibody

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling Nrf2 with purified ab62352 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody (1/1000) was used as the secondary antibody. Cells were counterstained with ab195889, anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594). DAPI was used to stain the nuclei blue. Secondary antibody only control: PBS was used instead of the primary antibody as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab62352).



Western blot - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

Nrf2 Western blot staining using rabbit Anti-Nrf2 antibody

This data was developed using ab62352, the same antibody clone in a different buffer formulation. False colour image of Western blot: Anti-Nrf2 antibody [EP1808Y] - ChIP Grade staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab62352 was shown to bind specifically to Nrf2.

Target band (indicated by arrow) was observed at 85-90 kDa in wild-type A549 cell lysates with no signal observed at this size in NFE2L2 knockout cell line ab285359 (knockout cell lysate ab289682). A band lower than the target was present on both WT and KO. According to the literature (PMID: 40043449), the lower band is calmegin.

To generate this image, wild-type and NFE2L2 knockout A549 cell lysates were analysed. Please note that MG132 treatment does not affect expression levels of Nrf2. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-Nrf2 antibody [EP1808Y] (ab62352) at 1/1000 dilution

Lane 1:
Wild-type A549 Vehicle control MG132 (0 uM, 18 h) cell lysate at 20 µg

Lane 2:
Wild-type A549 Treated MG132 (2 uM, 18 h) cell lysate at 20 µg

Lane 3:
NFE2L2 [21] knockout A549 Vehicle control MG132 (0 uM, 18 h) cell lysate at 20 µg

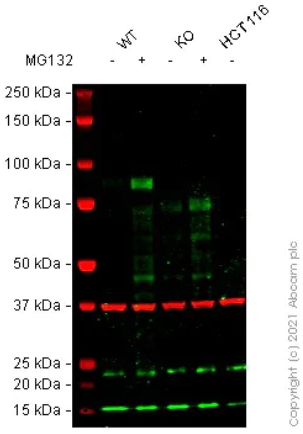
Lane 4:
NFE2L2 [21] knockout A549 Treated MG132 (2 uM, 18 h) cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 68 kDa



Western blot - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

Nrf2 Western blot staining using rabbit Anti-Nrf2 antibody

All lanes:
Western blot - Anti-Nrf2 antibody [EP1808Y] (ab62352) at 1/500 dilution

Lane 1:
Western blot - Human NFE2L2 (Nrf2) knockout HeLa cell lysate (ab263934)

Lane 1:
Wild-type HeLa control MG132 (0 uM, 18 h) cell lysate

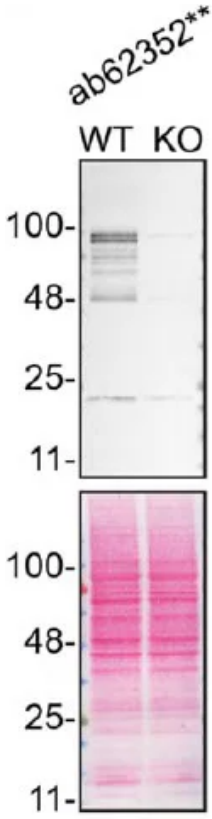
Lane 2:
Wild-type HeLa treated MG132 (2 uM, 18 h) cell lysate

Lane 2:
Western blot - Human NFE2L2 (Nrf2) knockout HeLa cell line (ab262507)

Lane 3:
NFE2L2 knockout HeLa control MG132 (0 uM, 18 h) cell lysate

Lane 4:
NFE2L2 knockout HeLa treated MG132 (2 uM, 18 h) cell lysate

Lane 5:
HCT 116 cell lysate



Western blot - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

Nrf2 Western blot staining using rabbit Anti-Nrf2 antibody

This data was developed using ab62352, the same antibody clone in a different buffer formulation.

ab62352 was shown to react with NFE2L2 in MG-132-treated wild-type HCT 116 cells in Western blot with loss of signal observed in MG-132-treated NFE2L2 knockout cell line ab287647. Wild-type HCT 116 and NFE2L2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab62352 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with secondary antibodies at 0.2 µg/mL before imaging.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.