abcam

Product datasheet

Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free ab180845

KO VALIDATED Recombinant RabMAb

13 References 14 Images

Overview

Product name	Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free
Description	Rabbit monoclonal [EP1808Y] to Nrf2 - BSA and Azide free
Host species	Rabbit
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).**
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, IHC-P, WB **Unsuitable for:** ChIP or IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab167152**)
Positive control	WB: MG-132 treated HeLa whole cell lysate, THP-1, MG-132 treated HepG2 whole cell lysate, MG-132 treated HCT-116 and A549 whole cell lysate. IHC-P: Human pancreatic carcinoma and kidney cancer tissues. ICC/IF: HepG2 and HeLa cells. Flow Cyt (intra): HeLa cells.
General notes	ab180845 is the carrier-free version of **ab62352**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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.

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 production

For more information **see here**.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1808Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab180845 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Predicted molecular weight: 68 kDa.

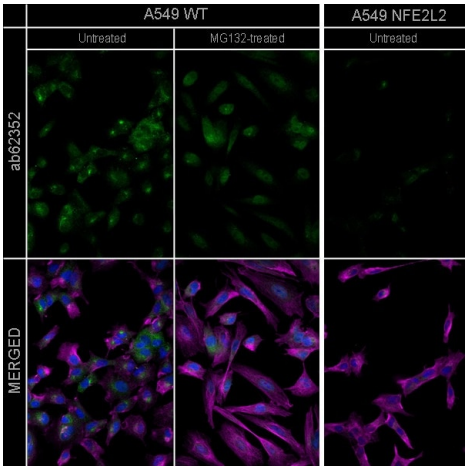
Application notes Is unsuitable for ChIP or IP.

Target

Function Transcription activator that binds to antioxidant response (ARE) elements in the promoter regions of target genes. Important for the coordinated up-regulation of genes in response to oxidative stress. May 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.

Tissue specificity	Widely expressed. Highest expression in adult muscle, kidney, lung, liver and in fetal muscle.
Sequence similarities	Belongs to the bZIP family. CNC subfamily. Contains 1 bZIP domain.
Domain	Acidic activation domain in the N-terminus, and DNA binding domain in the C-terminus.
Post-translational modifications	Phosphorylation of Ser-40 by PKC in response to oxidative stress dissociates NFE2L2 from its cytoplasmic inhibitor KEAP1, promoting its translocation into the nucleus.
Cellular localization	Cytoplasm > cytosol. Nucleus. Cytosolic under unstressed conditions, translocates into the nucleus upon induction by electrophilic agents.

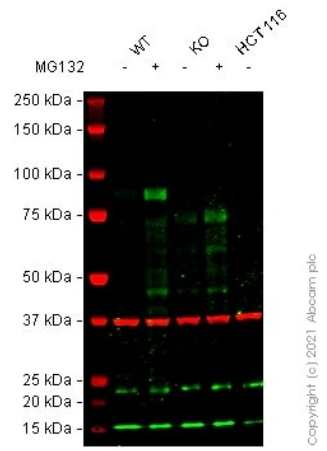
Images



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

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

ab62352 staining Nrf2 in untreated wild type A549 cells (left panel), treated wild type A549 cells (middle panel) and untreated NFE2L2 knockout A549 cells (right 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).



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

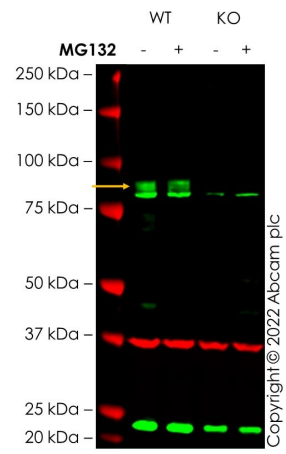
All lanes : Anti-Nrf2 antibody [EP1808Y] (**ab62352**) at 1/500 dilution

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 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

Lysates/proteins at 20 µg per lane.

Predicted band size: 68 kDa
Observed band size: 85 kDa

False colour image of Western blot: Anti-Nrf2 antibody [EP1808Y] - ChIP Grade staining at 1/500 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. A band was observed at 85 kDa in wild-type HeLa cell lysates with no signal observed at this size in NFE2L2 CRISPR-Cas9 edited cell line **ab262507** (CRISPR-Cas9 edited cell lysate **ab263934**). The band observed in the CRISPR-Cas9 edited lysate lane below 85 kDa is likely to represent a truncated form of Nrf2. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and NFE2L2 CRISPR-Cas9 edited HeLa cell lysates were analysed. 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 (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



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

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

Lane 1 : Wild-type A549 Vehicle control MG132 (0 uM, 18 h) cell lysate
Lane 2 : Wild-type A549 Treated MG132 (2 uM, 18 h) cell lysate
Lane 3 : NFE2L2 [21] knockout A549 Vehicle control MG132 (0 uM, 18 h) cell lysate
Lane 4 : NFE2L2 [21] knockout A549 Treated MG132 (2 uM, 18 h) cell lysate

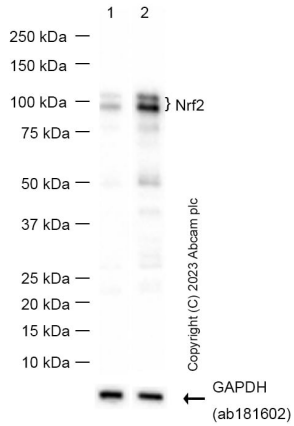
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 85-90 kDa

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. A band 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**). 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.



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

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

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate treated with MG-132 2uM for 18h

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 68 kDa
Observed band size: 955-110 kDa

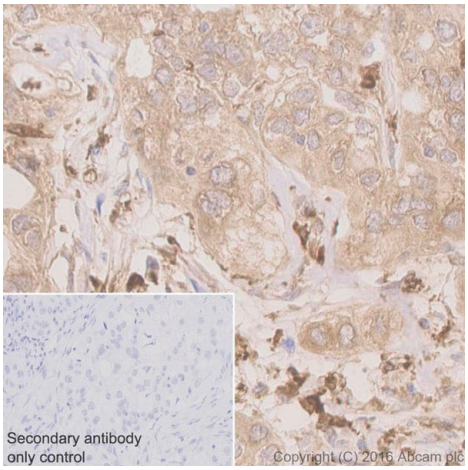
Exposure time: 10 seconds

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

Blocking and diluting buffer and concnetration: 5% NFDM/TBST.

ab181602 was used as GAPDH loading control.

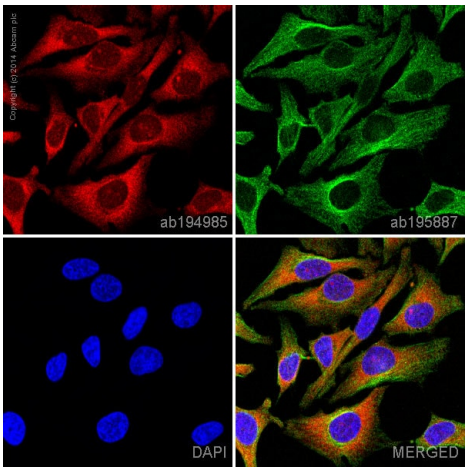
The two bands are different isoforms of Nrf2, the molecular weight observed is consistent with what has been described in the literature: PMID: 17512459, PMID: 22703241.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human pancreatic carcinoma tissue labelling Nrf2 with purified **ab62352** at a dilution of 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9 (**ab93684**). **Goat Anti-Rabbit IgG H&L (HRP) (ab97051)** was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

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



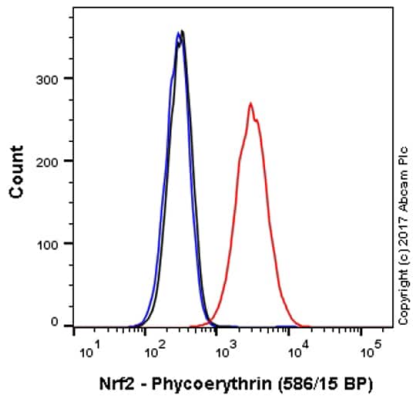
Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

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).



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

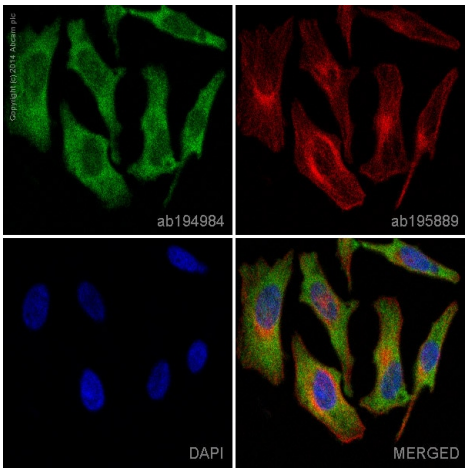
Clone EP1808Y (ab180845) has been successfully conjugated by Abcam. This image was generated using Anti-Nrf2 antibody [EP1808Y] (PE). Please refer to **ab223926** for protocol details.

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.

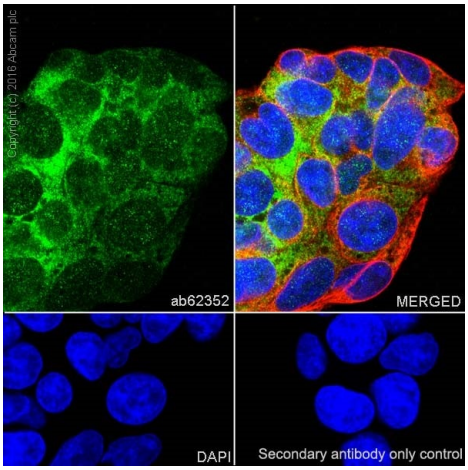


Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

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).

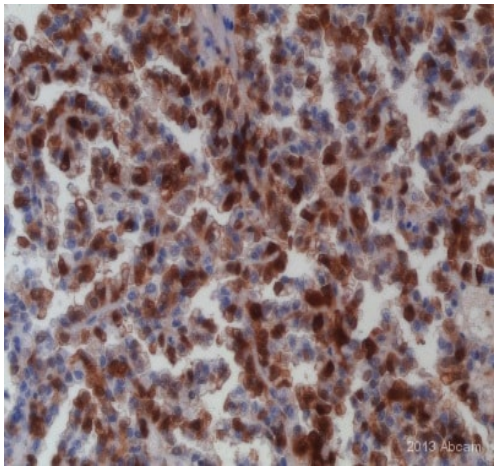


Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

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**).

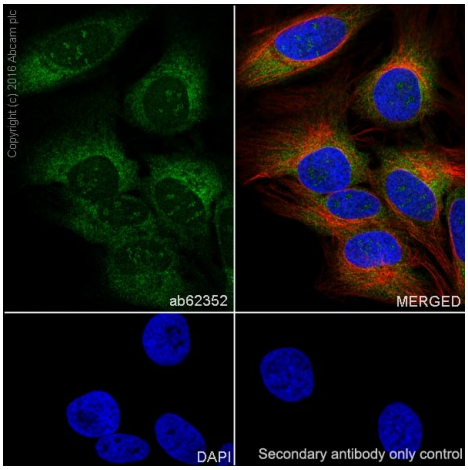


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

This image is courtesy of an Abreview submitted by Rudolf Jung.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney cancer tissue sections labeling Nrf2 with **ab62352** at 1/100 dilution. The tissue was fixed with paraformaldehyde and a heat mediated antigen retrival step was performed with TRIS-EDTA Buffer pH 9.0. Staining with **ab62352** at 1/100 was carried out in a dilution buffer with blocking for 30 minutes at 20°C. A undiluted goat anti-rabbit HRP conjugated secondary antibody was used.

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

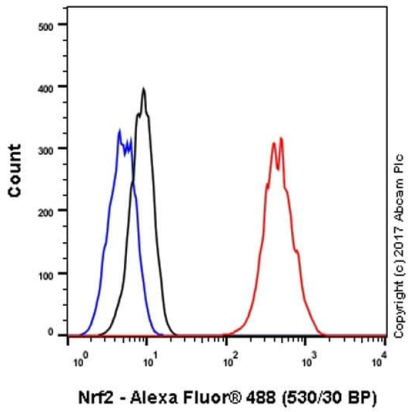


Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] - BSA and Azide free (ab180845)

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.

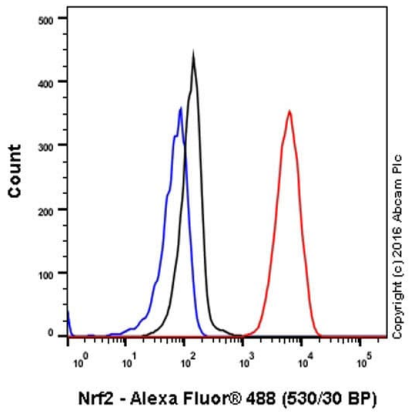
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab62352**).



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

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**).



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

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**).

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

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