abcam

Product datasheet

Anti-Nrf2 antibody [EP1808Y] ab62352

KO 評価済 リコンビナント RabMAb

32 Abreviews 490 References 画像数 13

製品の概要

製品名	Anti-Nrf2 antibody [EP1808Y]
製品の詳細	Rabbit monoclonal [EP1808Y] to Nrf2
由来種	Rabbit
特異性	**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).**
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, WB, IHC-P **適用なし:** ChIP or IP
種交差性	**交差種:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	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, Human iPSC-cardiomyocytes nuclear fraction IHC-P: Human pancreatic carcinoma and kidney cancer tissues. ICC/IF: HepG2 and MG-132 treated HeLa cells. Flow Cyt (intra): HeLa cells.
特記事項	PLEASE NOTE: Nrf2 antibody (ab62352) detects no signal in most untreated samples for WB. Stimuli treated samples are recommended. Nrf2 expression is stimulated by oxidative stress, electrophiles and chemical activators (PMID: 25761198, PMID: 27638861 and PMID: 28587109) 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**.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EP1808Y
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab62352の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/40.
ICC/IF	★★★★★ (4)	1/1000 - 1/5000.
WB	★★★★☆ (24)	1/200 - 1/1000. Predicted molecular weight: 68 kDa.
IHC-P	★★★★★ (2)	1/100 - 1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

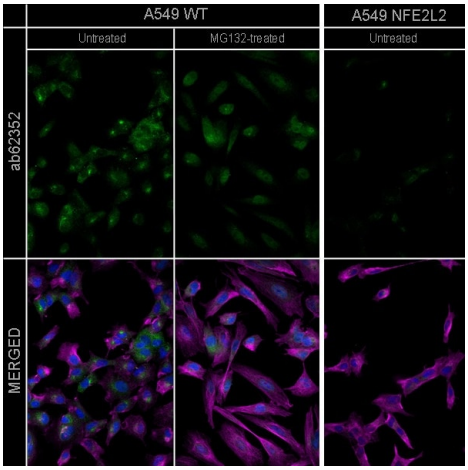
追加情報 Is unsuitable for ChIP or IP.

ターゲット情報

機能	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.
組織特異性	Widely expressed. Highest expression in adult muscle, kidney, lung, liver and in fetal muscle.
配列類似性	Belongs to the bZIP family. CNC subfamily. Contains 1 bZIP domain.
ドメイン	Acidic activation domain in the N-terminus, and DNA binding domain in the C-terminus.

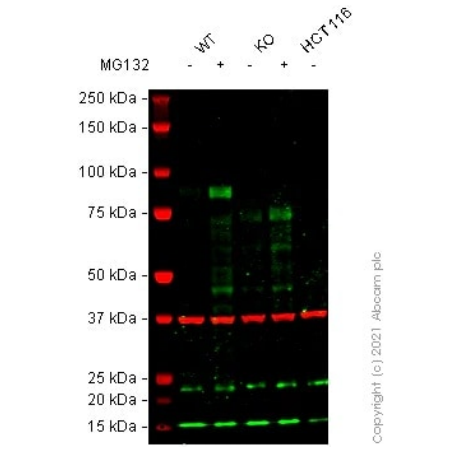
翻訳後修飾	Phosphorylation of Ser-40 by PKC in response to oxidative stress dissociates NFE2L2 from its cytoplasmic inhibitor KEAP1, promoting its translocation into the nucleus.
細胞内局在	Cytoplasm > cytosol. Nucleus. Cytosolic under unstressed conditions, translocates into the nucleus upon induction by electrophilic agents.

画像



Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] (ab62352)

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] (ab62352)

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

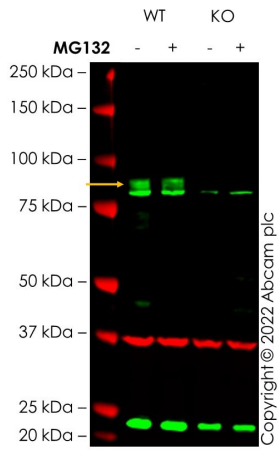
Lane 1 : Wild-type HeLa control MG132 (0 uM, 18 h) cell lysate
Lane 2 : Wild-type HeLa treated MG132 (2 uM, 18 h) cell lysate
Lane 3 : NFE2L2 knockout HeLa control MG132 (0 uM, 18 h) cell lysate
Lane 4 : NFE2L2 knockout HeLa treated MG132 (2 uM, 18 h) cell lysate
Lane 5 : HCT 116 cell lysate

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] (ab62352)

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.

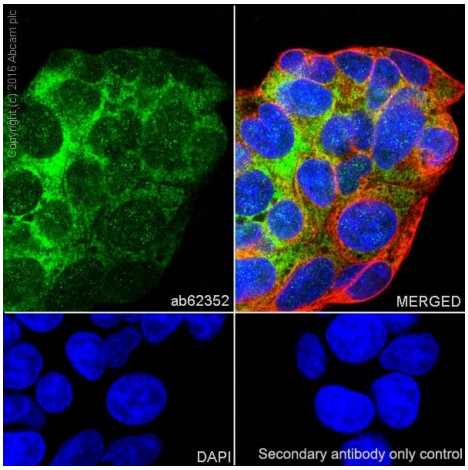
Predicted band size: 68 kDa
Observed band size: 85-90 kDa

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, we are unsure about the identity of this band.

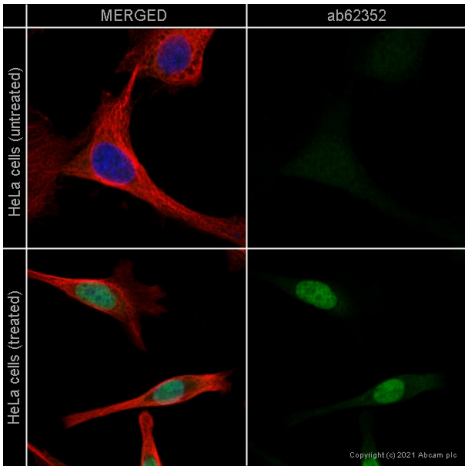
To generate this image, wild-type and NFE2L2 knockout A549 cell lysates were analysed. Please note that MG132 treatment does not affect expression levels of Nrf2. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % 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.



Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] (ab62352)

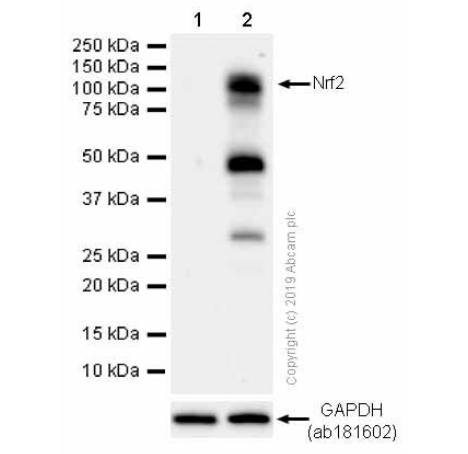
Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling Nrf2 with purified ab62352 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. 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.



Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] (ab62352)

ab62352 staining Nrf2 in untreated HeLa cells (top panel) and treated HeLa cells (bottom 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 1µg/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 red). 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] (ab62352)

All lanes : Anti-Nrf2 antibody [EP1808Y] (ab62352) at 1/200 dilution (Purified)

Lane 1 : HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST
Lane 2 : HCT 116 (Human colorectal carcinoma epithelial cell) treated with 25uM MG-132 for 4 hours whole cell lysates with 5% NFDM/TBST

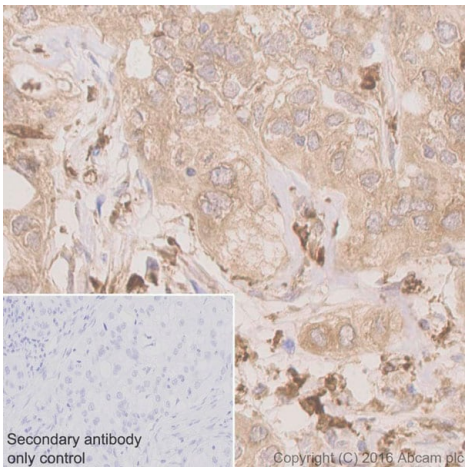
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 68 kDa
Observed band size: 100 kDa

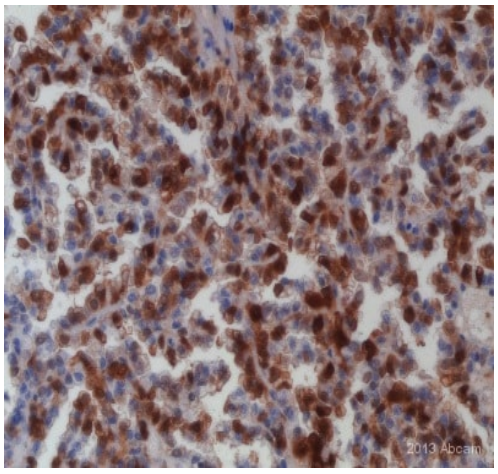
Exposure time: 60 seconds

The expression of Nrf2 is stimulated by oxidative stress, electrophiles and chemical activators (PMID: 25761198, PMID: 27638861 and PMID: 28587109). ab62352 detects no signal in most of the untreated samples in WB. Stimuli treated samples are recommended.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nrf2 antibody [EP1808Y] (ab62352)

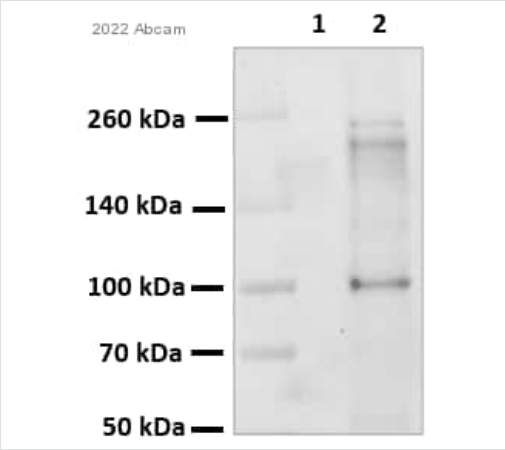
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human pancreatic carcinoma tissue labelling Nrf2 with purified ab62352 at a dilution of 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9 (**ab93684**). **Goat Anti-Rabbit IgG H&L (HRP) (ab97051)**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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nrf2 antibody [EP1808Y] (ab62352)

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.



Western blot - Anti-Nrf2 antibody [EP1808Y] (ab62352)

This image is courtesy of an Abreview submitted by Marcos Castro Guarda

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

Lane 1 : Human iPSC-cardiomyocytes cytoplasmatic fraction
Lane 2 : Human iPSC-cardiomyocytes nuclear fraction

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab6721**) at 1/2000 dilution

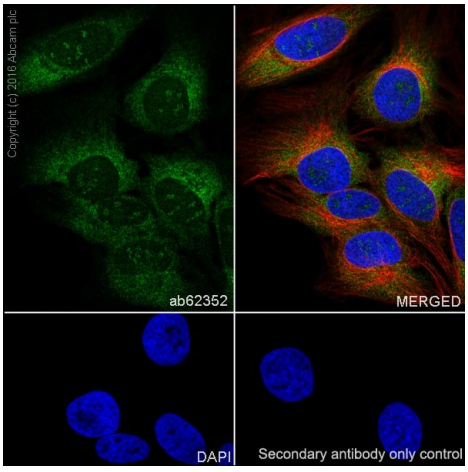
Developed using the ECL technique.

Predicted band size: 68 kDa
Observed band size: 100 kDa

Exposure time: 1 minute

Western blot analysis using ab62352 at 1:1000 on Human iPSC-cardiomyocytes. Blocking agent and dilution buffer was 5% Skim Milk in TBS-Tween.

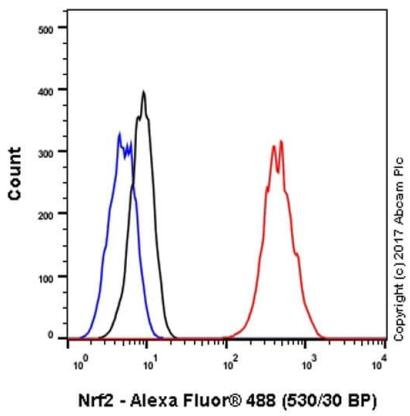
A cellular fractionation in Human iPSC-cardiomyocyte cells was performed to separate the nucleus from the cytoplasm (Lane 2).



Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 antibody [EP1808Y] (ab62352)

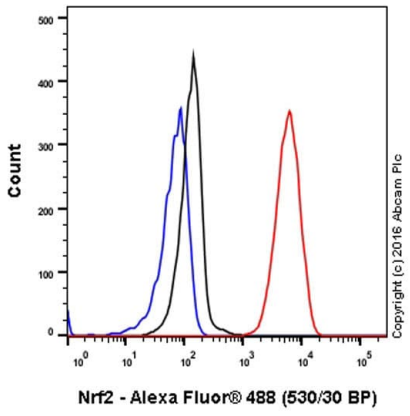
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling Nrf2 with purified ab62352 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody** (1/1000) was used as the secondary 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.



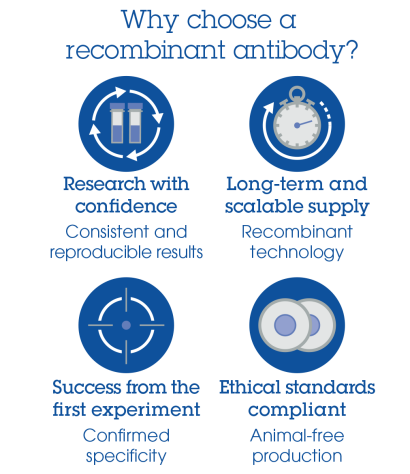
Flow Cytometry (Intracellular) - Anti-Nrf2 antibody [EP1808Y] (ab62352)

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.



Flow Cytometry (Intracellular) - Anti-Nrf2 antibody [EP1808Y] (ab62352)

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 Fluorr®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.



Anti-Nrf2 antibody [EP1808Y] (ab62352)

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