Product name: Anti-Nrf2 (phospho S40) antibody [EP1809Y] ab76026

Description: Rabbit monoclonal [EP1809Y] to Nrf2 (phospho S40)

Host species: Rabbit

Tested applications: Suitable for: Dot blot, ICC/IF, IHC-P, WB, Flow Cyt
Unsuitable for: IP

Species reactivity: Reacts with: Human

Immunogen: Synthetic peptide within Human Nrf2 (phospho S40). The exact sequence is proprietary.
(Peptide available as ab133404)


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

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

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

Form: Liquid


Storage buffer: pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity: Protein A purified
Clonality  Monoclonal
Clone number  EP1809Y
Isotype  IgG

Applications

Our Abpromise guarantee covers the use of ab76026 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<th>Abreviews</th>
<th>Notes</th>
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<td>Dot blot</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>ICC/IF</td>
<td>1/100 - 1/250.</td>
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<tr>
<td>IHC-P</td>
<td>1/100 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.</td>
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<tr>
<td>Flow Cyt</td>
<td>1/80 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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Application notes  Is unsuitable for 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
Nrf2 was abundantly expressed in carcinomas, low grade dysplasias, and non-atypical epithelia of oral tissue.

Representative findings of Nrf2 staining in carcinoma (left), in low grade dysplasia (middle), and in non-atypical epithelium (right).

Corresponding PLA signals are displayed in the lower row. Scale bar; 100 µm.

Surgical specimens were transferred to 10% buffered formalin and fixed overnight. The fixed samples were embedded in paraffin, and serially sliced into 5 µm sections. After dewaxing, sections were autoclaved at 120°C for 1 min in 10 mM sodium citrate buffer (pH 6.0), and immersed in 0.3% H₂O₂. They were then incubated overnight at 4°C with primary antibody to Nrf2 (diluted 1:200). The sections were rinsed with 1×PBS and incubated with the secondary antibody conjugated with horseradish peroxidase at room temperature for 1 hour. The sections were then stained with 3,3′-diaminobenzidinetetrahydrochloride (DAB) and counterstained with hematoxylin.

Immunofluorescence analysis of Nrf2 levels in Kaposi’s sarcoma skin lesions.

B) Healthy skin (top two rows) and KS skin tissue (bottom row) were double-stained for LANA-1 (Alexa-Fluor 594- red) and host phosphorylated pNrf2 (ab76026) (Alexa-Fluor®488 – green). DAPI was used to visualize the nuclei, and the triple merge of LANA-1, pNrf2 and DAPI is shown in the third column. Yellow square=enlarged area.
**Nrf2 Translocation from cytoplasm to nucleus.**

(A) Human islets were treated with dh404 for 0.5, 1 or 2 hours. The treated and untreated samples were stained with Nrf2 antibody ab76026 (Green) and DAPI (Blue). The con-focal microscope clearly showed that the Nrf2 translocation from cytoplasm to nucleus in the dh404 treated human islet cells.

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**Immunocytochemistry/ Immunofluorescence - Anti-Nrf2 (phospho S40) antibody [EP1809Y] (ab76026)**


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**Western blot - Anti-Nrf2 (phospho S40) antibody [EP1809Y] (ab76026)**

All lanes: Anti-Nrf2 (phospho S40) antibody [EP1809Y] (ab76026) at 1/50000 dilution (purified)

Lane 1: untreated HepG2 cell lysate
Lane 2: HepG2 treated with phosphatase lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP goat anti-rabbit IgG (H+L) at 1/20000 dilution

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

why is the actual band size different from the predicted?

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
Immunohistochemical staining of paraffin embedded human breast carcinoma with purified ab76026 at a working dilution of 1/500. The secondary antibody used is HRP goat anti-rabbit IgG H&L (ab97051) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

Immunofluorescence staining of HepG2 cells with purified ab76026 at a working dilution of 1/100, counter-stained with DAPI. The treated cells were treated with alkaline phosphatase for 1 h at 37°C. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab76026 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.

Overlay histogram showing Jurkat cells fixed in 4% PFA and stained with purified ab76026 at a dilution of 1 in 80 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).
Dot blot analysis of Nrf2 peptides using unpurified ab76026 at 1/1000 dilution followed by Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated secondary antibody at 1/1000 dilution. Blocking and diluting buffer was 5% NFDM/TBST.

Lane 1: Nrf2 (pS40) phospho peptide
Lane 2: Nrf2 non-phospho peptide

Immunohistochemical analysis of paraffin-embedded human breast carcinoma using unpurified ab76026 at 1/100 dilution.

All lanes: Anti-Nrf2 (phospho S40) antibody [EP1809Y] (ab76026) at 1/10000 dilution (unpurified)

Lane 1: Untreated HepG2 (human hepatocellular carcinoma) whole cell lysates 20µg
Lane 2: HepG2 (human hepatocellular carcinoma) treated with Alkaline Phosphatase (AP) whole cell lysates 20µg.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

Predicted band size: 68 kDa
Observed band size: 100 kDa why is the actual band size different from the predicted?

Blocking buffer: 5% NFDM/TBST, dilution buffer: 5% NFDM/TBST, exposure time: 15 seconds
Western blot - Anti-Nrf2 (phospho S40) antibody [EP1809Y] (ab76026)

All lanes: Anti-Nrf2 (phospho S40) antibody [EP1809Y] (ab76026) at 1/20000 dilution (unpurified)

Lane 1: HepG2 cell lysate
Lane 2: HepG2 cell lysate treated with AP

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: HRP labelled goat anti-rabbit at 1/1000 dilution

Predicted band size: 68 kDa
Observed band size: 90 kDa why is the actual band size different from the predicted?

Unpurified ab76026 staining Nrf2 (phospho S40) in Human normal lung tissue sections by IHC-P (Formaldehyde-fixed paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 1% casein for 30 minutes at 4°C. Antigen retrieval was by heat mediation. Samples were incubated with primary antibody (1/50) in 1% casein for 24 hours at 4°C. An undiluted HRP-conjugated Goat polyclonal to rabbit IgG was used as the secondary antibody.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nrf2 (phospho S40) antibody [EP1809Y] (ab76026)

Unpurified ab76026 showing positive staining in Breast carcinoma tissue.

Unpurified ab76026 showing positive staining in Cervical carcinoma tissue.

Unpurified ab76026 showing positive staining in Ovarian carcinoma tissue.
Unpurified ab76026 showing positive staining in Normal tonsil tissue.

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