abcam

Product datasheet

Anti-GCN2 antibody [EPR5970(2)] ab134053

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-GCN2 antibody [EPR5970(2)]
Description	Rabbit monoclonal [EPR5970(2)] to GCN2
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, Flow Cyt, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide corresponding to Human GCN2 (N terminal).
Positive control	HeLa, 293T, MOLT4, MCF7 and A549 cell lysates; Human kidney and human breast carcinoma tissue.
General notes	Mouse, 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.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR5970(2)
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab134053** in the following tested applications.

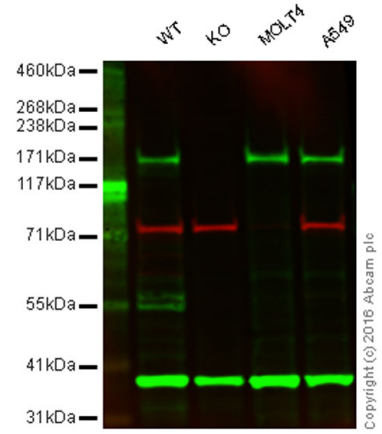
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆	1/1000 - 1/10000. Detects a band of approximately 220 kDa (predicted molecular weight: 187 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
Flow Cyt		1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/1000 - 1/10000
ICC/IF		1/250 - 1/500.

Target

Function	Can phosphorylate the alpha subunit of EIF2 and may mediate translational control.
Tissue specificity	Widely expressed.
Sequence similarities	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. GCN2 subfamily. Contains 2 protein kinase domains. Contains 1 RWD domain.
Domain	Kinase domain 1 is a degenerate kinase domain. RWD domain is reported to interact with GCN1L1.
Post-translational modifications	Autophosphorylated on threonine residues.

Images

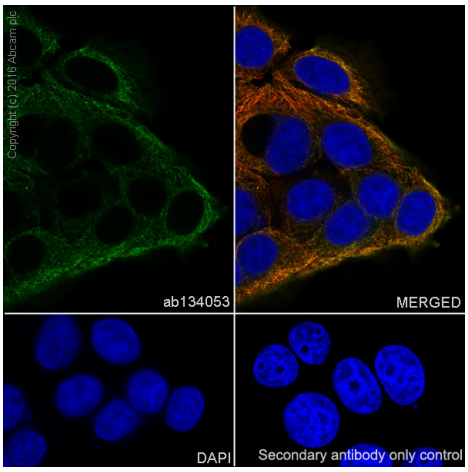


Western blot - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: GCN2 knockout HAP1 cell lysate (20 µg)
Lane 3: MOLT4 cell lysate (20 µg)
Lane 4: A549 cell lysate (20 µg)

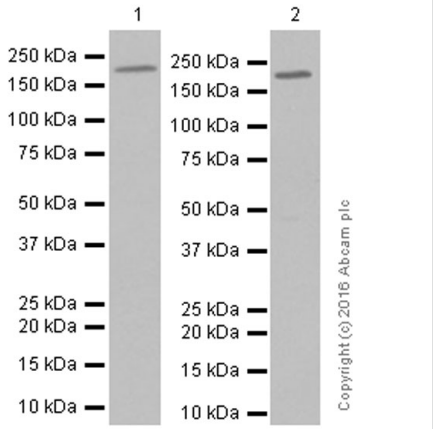
Lanes 1 - 4: Merged signal (red and green). Green - ab134053 observed at 171 kDa. Red - loading control, ab18058, observed at 124 kDa.

Unpurified ab134053 was shown to recognize GCN2 when GCN2 knockout samples were used, along with additional cross-reactive bands. Wild-type and GCN2 knockout samples were subjected to SDS-PAGE. ab134053 and ab18058 (loading control to Vinculin) were diluted at 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma cell line) cells labeling GCN2 with ab134053 at 1/250 dilution (4.0µg/ml). The cells were co-stained with ab195889, an Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 (2.5 µg/ml). Cells were fixed with 100% methanol. ab150077, a Goat anti-rabbit IgG(Alexa Fluor® 488) secondary antibody was used at 1/1000 dilution. DAPI was used as the nuclear counter stain.



Western blot - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

All lanes : Anti-GCN2 antibody [EPR5970(2)] (ab134053) at 1/10000 dilution (purified)

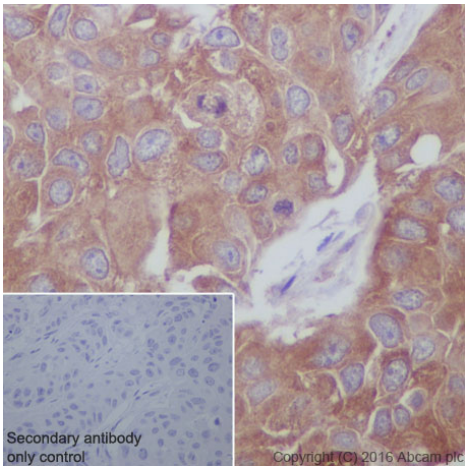
Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

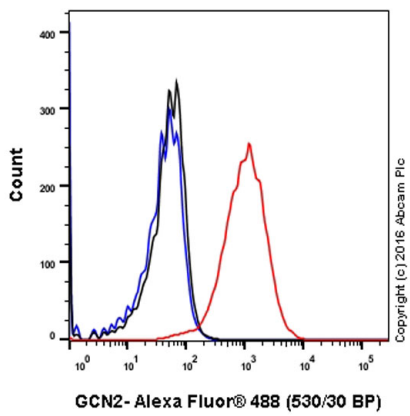
Predicted band size: 187 kDa
Observed band size: 187 kDa

Blocking and diluting buffer: 5% NFDM/TBST.



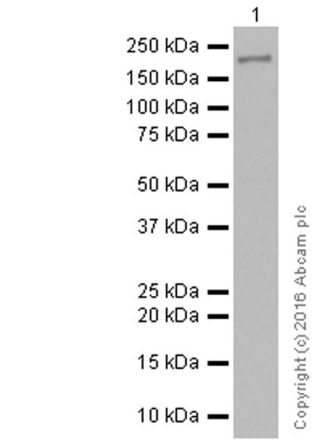
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carconoma tissue sections labeling GCN2 with purified ab134053 at 1/100 dilution (10 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, PH9. ab97051, a Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1/500 dilution. Tissue was counterstained with hematoxylin. PBS instead of the primary antibody was used as the negative control.



Flow Cytometry - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma cell line) cells labeling GCN2 with purified ab134053 at 1/100 dilution (10 ug/ml). Cells were fixed with 4% paraformaldehyde. A Goat anti-rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Rabbit monoclonal IgG (Black) was used as the isotypre control. Cells without incubation with the primary antibody and secondary antibody (Blue) is the unlabeled control.



Western blot - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

Anti-GCN2 antibody [EPR5970(2)] (ab134053) at 1/50000 dilution (purified) + MOLT-4 (Human lymphoblastic leukemia cell line) whole cell lysate at 20 µg

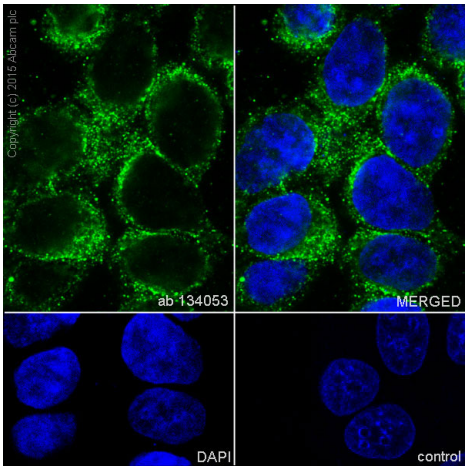
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 187 kDa

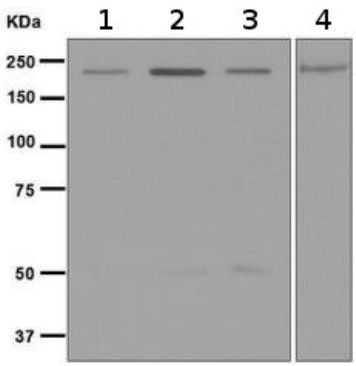
Observed band size: 187 kDa

Blocking and diluting buffer: 5% NFDM /TBST.



Immunocytochemistry/ Immunofluorescence - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

Immunofluorescence staining of MCF-7 cells with purified ab134053 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was an Alexa Fluor® 488 conjugated goat anti-rabbit (ab150077), used at a dilution of 1/1000. The cells were fixed in 100% methanol. The negative control is shown in bottom right hand panel - for the negative control, PBS was used instead of the primary antibody.



Western blot - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

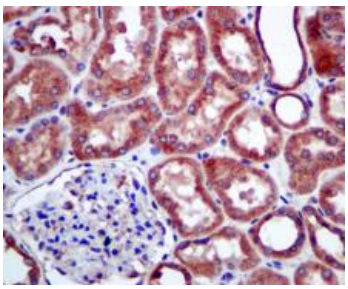
All lanes : Anti-GCN2 antibody [EPR5970(2)] (ab134053) at 1/1000 dilution (Unpurified)

Lane 1 : HeLa cell lysate
Lane 2 : 293T cell lysate
Lane 3 : MOLT4 cell lysate
Lane 4 : A549 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

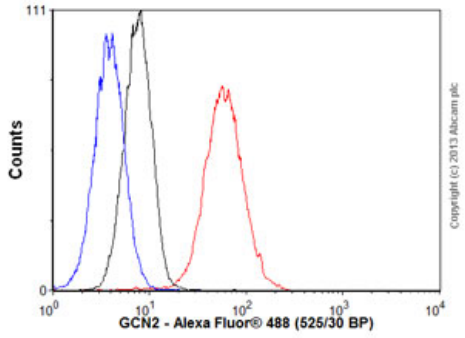
Predicted band size: 187 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labelling GCN2 with unpurified ab134053 at 1/100 dilution.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Flow Cytometry - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

Overlay histogram showing HeLa cells stained with unpurified ab134053 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab134053, 1/10000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

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