abcam

ab157775

Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free

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	EPR5970(2)
Purification technique	Affinity purification Protein A
Concentration	0.992 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-

Notes	-

Not recommended

Species	Rat, Mouse
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Rat, Mouse
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Rat, Mouse
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Rat, Mouse
Dilution info	-
Notes	-

IP

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

Target data

See full target information EIF2AK4

Function

Metabolic-stress sensing protein kinase that phosphorylates the alpha subunit of eukaryotic translation initiation factor 2 (EIF2S1/eIF-2-alpha) in response to low amino acid availability (PubMed:25329545, PubMed:32610081). Plays a role as an activator of the integrated stress response (ISR) required for adaptation to amino acid starvation (By similarity). EIF2S1/eIF-2-alpha phosphorylation in response to stress converts EIF2S1/eIF-2-alpha into a global protein synthesis inhibitor, leading to a global attenuation of cap-dependent translation, and thus to a reduced overall utilization of amino acids, while concomitantly initiating the preferential translation of ISR-specific mRNAs, such as the transcriptional activator ATF4, and hence allowing ATF4-mediated reprogramming of amino acid biosynthetic gene expression to alleviate nutrient depletion (PubMed:32610081). Binds uncharged tRNAs (By similarity). Required for the translational induction of protein kinase PRKCH following amino acid starvation (By similarity). Involved in cell cycle arrest by promoting cyclin D1 mRNA translation repression after the unfolded protein response pathway (UPR) activation or cell cycle inhibitor CDKN1A/p21 mRNA translation activation in response to amino acid deprivation (PubMed:26102367). Plays a role in the consolidation of synaptic plasticity, learning as well as formation

of long-term memory (By similarity). Plays a role in neurite outgrowth inhibition (By similarity). Plays a proapoptotic role in response to glucose deprivation (By similarity). Promotes global cellular protein synthesis repression in response to UV irradiation independently of the stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) and p38 MAPK signaling pathways (By similarity). Plays a role in the antiviral response against alphavirus infection; impairs early viral mRNA translation of the incoming genomic virus RNA, thus preventing alphavirus replication (By similarity). (Microbial infection) Plays a role in modulating the adaptive immune response to yellow fever virus infection; promotes dendritic cells to initiate autophagy and antigene presentation to both CD4(+) and CD8(+) T-cells under amino acid starvation (PubMed:24310610).

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

ab157775 is the carrier-free version of ab134053.

Species reactivity

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

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.

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

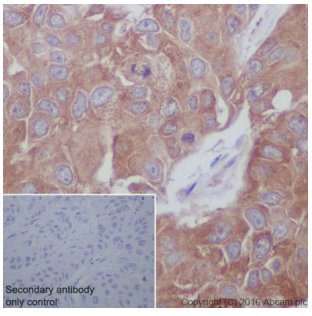
We do not recommend this combination. It is not covered by our product promise.

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.

10 product images

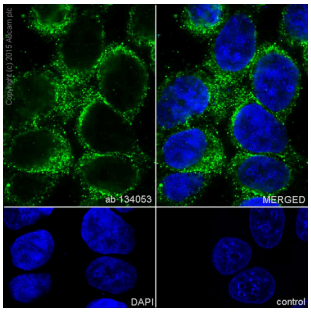


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free (ab157775)

GCN2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-GCN2 antibody

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.

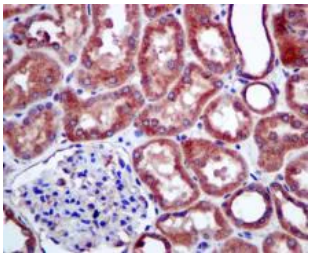
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134053).



Immunocytochemistry/ Immunofluorescence - Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free (ab157775)

GCN2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GCN2 antibody

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134053).

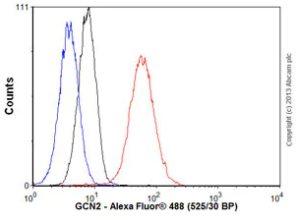


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free (ab157775)

GCN2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-GCN2 antibody

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labelling GCN2 with unpurified ab134053 at 1/100 dilution.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134053).

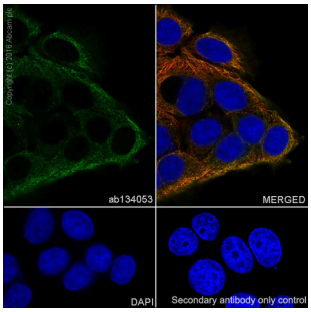
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free (ab157775)

GCN2 Flow Cytometry (Intracellular) staining using rabbit Anti-GCN2 antibody

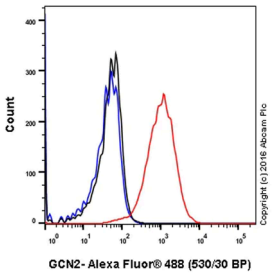
Overlay histogram showing HeLa cells stained with unpurified ab134053 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein 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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134053).



Immunocytochemistry/ Immunofluorescence - Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free (ab157775)

GCN2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GCN2 antibody

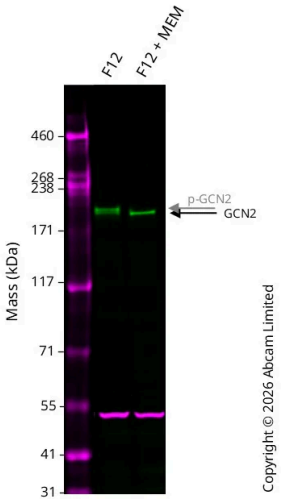
Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma cell line) cells labeling GCN2 with ab134053 at 1/250 dilution (4.0µg/ml). The cells were co-stained with ab195889, an Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 (2.5 µg/ml). 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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134053).



Flow Cytometry (Intracellular) - Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free (ab157775)

GCN2 Flow Cytometry (Intracellular) staining using rabbit Anti-GCN2 antibody

Intracellular 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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134053).



Western blot - Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free (ab157775)

GCN2 Western blot staining using rabbit Anti-GCN2 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134053).

Western blot: Anti-GCN2 antibody [EPR5970(2)] ab134053 staining at 1/1000 dilution, shown in green; Mouse anti alpha Tubulin ab7291 loading control staining at 1/20000 dilution, shown in magenta. Phosphorylated GCN2 runs at an apparantly higher molecular weight than unphosphorylated GCN2 after amino acid deprivation. To generate this image, 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 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-GCN2 antibody [EPR5970(2)] (ab134053) at 1/1000 dilution

Lane 1:
A549, amino acid deprivation: F12 media without MEM cell lysates at 20 µg

Lane 2:
A549, control: F12 supplemented with MEM cell lysates at 20 µg

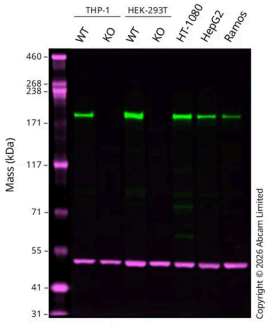
Secondary

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

Performed under reducing conditions.

Predicted band size: 187 kDa

Observed band size: 187-200 kDa



Western blot - Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free (ab157775)

GCN2 Western blot staining using rabbit Anti-GCN2 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134053).

Western blot: Anti-GCN2 antibody [EPR5970(2)] ab134053 staining at 1/1000 dilution, shown in green; Mouse anti alpha Tubulin ab7291 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 187 kDa in Wild-type THP-1 cell lysates with no signal observed at this size in EIF2AK4 knockout THP-1 cell line. To generate this image, 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 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-GCN2 antibody [EPR5970(2)] (ab134053) at 1/1000 dilution

Lane 1:
Wild-type THP-1 cell lysates at 20 µg

Lane 2:
Western blot - Human EIF2AK4 knockout THP1 cell line (ab290213) at 20 µg

Lane 3:
Wild-type HEK-293T ab255449 cell lysates at 20 µg

Lane 4:
Western blot - Human EIF2AK4 (GCN2) knockout HEK-293T cell line (ab267247) at 20 µg

Lane 5:
HT1080 cell lysates at 20 µg

Lane 6:
HepG2 cell lysates at 20 µg

Lane 7:
Ramos cell lysates at 20 µg

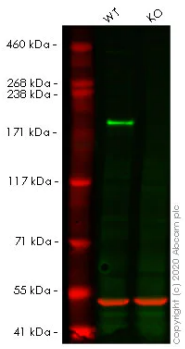
Secondary

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

Performed under reducing conditions.

Predicted band size: 187 kDa

Observed band size: 187 kDa



Western blot - Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free (ab157775)

GCN2 Western blot staining using rabbit Anti-GCN2 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134053).
Lanes 1- 2: Merged signal (red and green). Green - ab134053 observed at 187 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) observed at 50 kDa.

ab134053 was shown to react with GCN2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab267247 (knockout cell lysate ab256903) was used. Wild-type HEK-293T and EIF2AK4 knockout HEK-293T cell lysates were subjected to SDS-PAGE. ab134053 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.

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

Lane 1:
Wild-type HEK-293T cell lysate at 20 µg

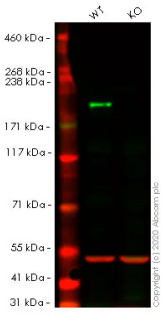
Lane 2:
EIF2AK4 knockout HEK-293T cell lysate at 20 µg

Lane 2:
Western blot - Human EIF2AK4 (GCN2) knockout HEK-293T cell line (ab267247)

Performed under reducing conditions.

Predicted band size: 112 kDa, 13 kDa, 187 kDa, 55 kDa, 69 kDa

Observed band size: 187 kDa



Western blot - Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free (ab157775)

GCN2 Western blot staining using rabbit Anti-GCN2 antibody

This data was developed using the same antibody clone in a different buffer formation (ab134052).
Lanes 1- 2: Merged signal (red and green). Green - ab134053 observed at 187 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) observed at 50 kDa.

ab134053 was shown to react with GCN2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab267246 (knockout cell lysate ab256902) was used. Wild-type HEK-293T and EIF2AK4 knockout HEK-293T cell lysates were subjected to SDS-PAGE. ab134053 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye800®CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

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

Lane 1:

Wild-type HEK-293T cell lysate at 20 µg

Lane 2:
Western blot - Human EIF2AK4 (GCN2) knockout HEK-293T cell lysate (ab256902) at 20 µg

Performed under reducing conditions.

Predicted band size: 187 kDa

Observed band size: 187 kDa

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