abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

9 Images

Overview

Product name	Anti-GCN2 antibody [EPR5970(2)] - BSA and Azide free
Description	Rabbit monoclonal [EPR5970(2)] to GCN2 - BSA and Azide free
Host species	Rabbit
Specificity	This antibody does not react with mouse and rat species in Western blot application.
Tested applications	**Suitable for:** IHC-P, Flow Cyt (Intra), ICC/IF, WB **Unsuitable for:** IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK293T cell lysate. Flow Cyt (intra): MCF7 and HeLa cells. ICC/IF: MCF7 cells. IHC-P: Human breast carcinoma tissue.
General notes	ab157775 is the carrier-free version of **ab134053**. 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**.

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

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5970(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab157775 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
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 220 kDa (predicted molecular weight: 187 kDa).

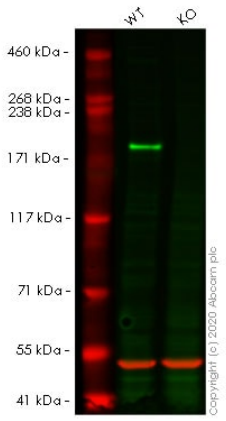
Application notes Is unsuitable for IP.

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)] - BSA and Azide free (ab157775)

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

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : EIF2AK4 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

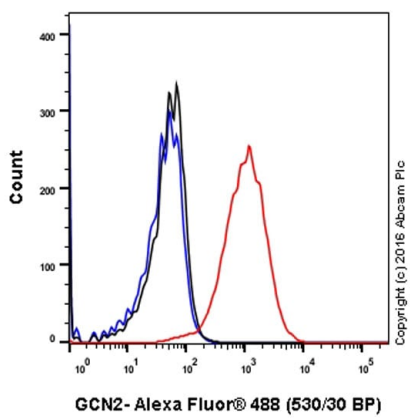
Performed under reducing conditions.

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

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.

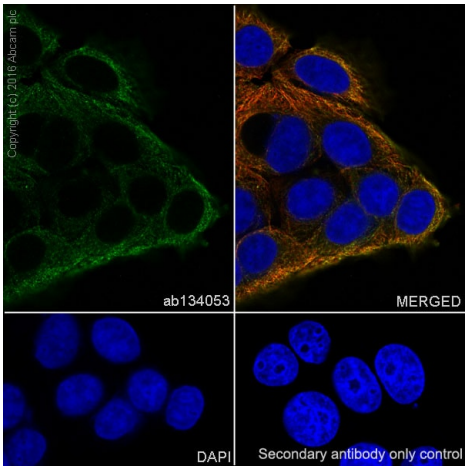
ab134053 was shown to react with GCN2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab267247** (knockout cell lysate **ab256903**) was used. Wild-type HEK-293T and EIF2AK4 knockout HEK-293T cell lysates were subjected to SDS-PAGE. **ab134053** and 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.



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

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.

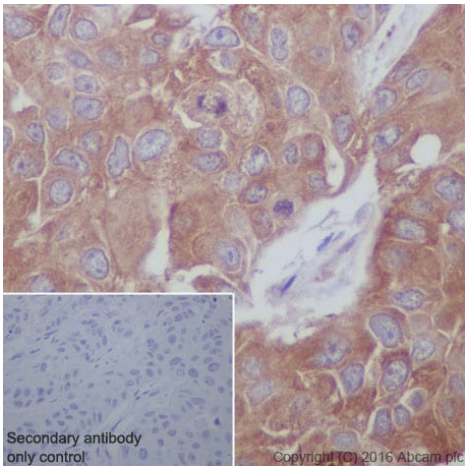
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab134053**).



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

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.

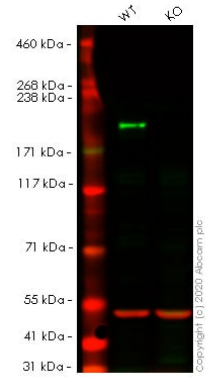
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab134053**).



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

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**).



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

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

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : EIF2AK4 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

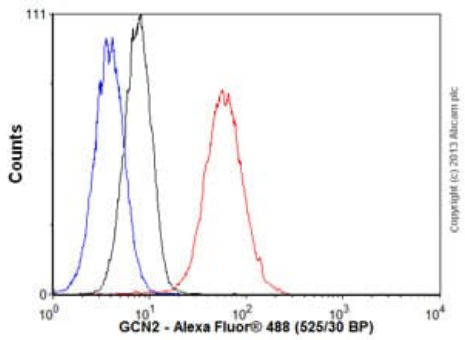
Predicted band size: 187 kDa
Observed band size: 187 kDa

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)

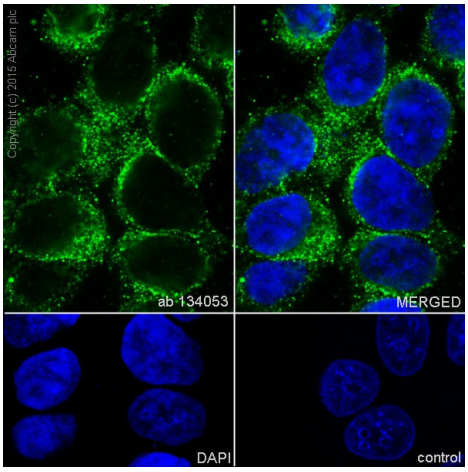
preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



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

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.

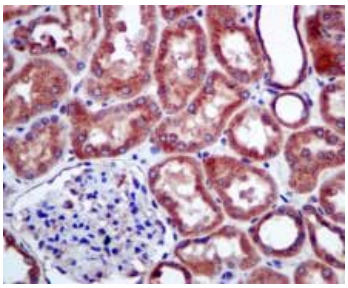
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab134053**).



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

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)

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.

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

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

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.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors