abcam

Product datasheet

Anti-GCN2 antibody [EPR5970(2)] ab134053

KO 評価済 リコンビナント RabMAb

2 Abreviews 9 References 画像数 13

製品の概要

製品名	Anti-GCN2 antibody [EPR5970(2)]
製品の詳細	Rabbit monoclonal [EPR5970(2)] to GCN2
由来種	Rabbit
特異性	This antibody does not react with mouse and rat species in Western blot application.
アプリケーション	**適用あり:** WB, IHC-P, Flow Cyt (Intra), ICC/IF **適用なし:** IP
種交差性	**交差種:** Human
免疫原	Synthetic peptide corresponding to Human GCN2 (N terminal).
ポジティブ・コントロール	WB: HEK-293T, HAP1, HeLa, 293T, MOLT4, MCF7 and A549 cell lysates. ICC/IF: MCF7 cells. Flow Cyt (intra): MCF7 and HeLa cells. IHC-P: Human kidney and human breast carcinoma tissue.
特記事項	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.

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 long term. Avoid freeze / thaw cycle. Stable for 12 months at -20°C.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 0.05% BSA, 40% Glycerol, 59% PBS
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR5970(2)
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ab134053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。

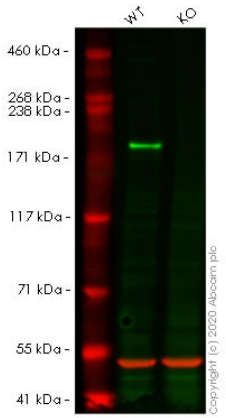
アプリケーション	Abreviews	特記事項
WB	★★★★☆ (2)	1/1000 - 1/10000. Detects a band of approximately 220 kDa (predicted molecular weight: 187 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
Flow Cyt (Intra)		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.

追加情報 Is unsuitable for IP.

ターゲット情報

機能	Can phosphorylate the alpha subunit of EIF2 and may mediate translational control.
組織特異性	Widely expressed.
配列類似性	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. GCN2 subfamily. Contains 2 protein kinase domains. Contains 1 RWD domain.
ドメイン	Kinase domain 1 is a degenerate kinase domain. RWD domain is reported to interact with GCN1L1.
翻訳後修飾	Autophosphorylated on threonine residues.

画像



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

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

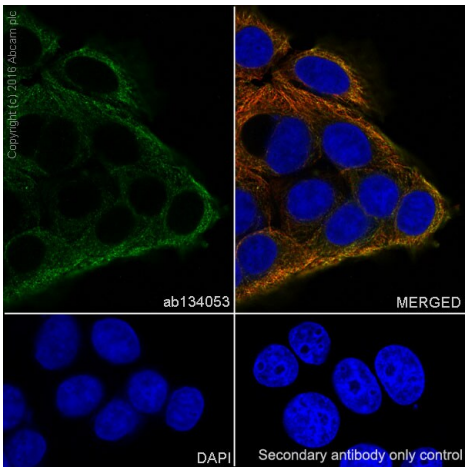
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

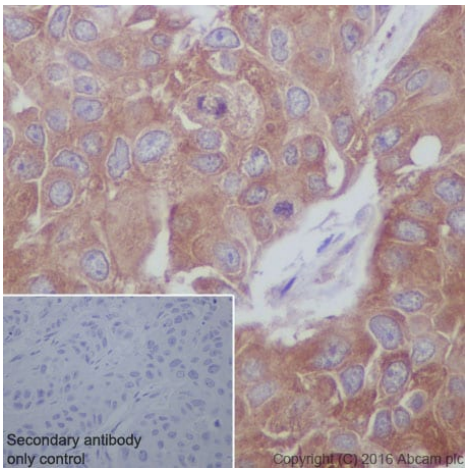
Lanes 1- 2: Merged signal (red and green). Green - ab134053 observed at 187 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

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.



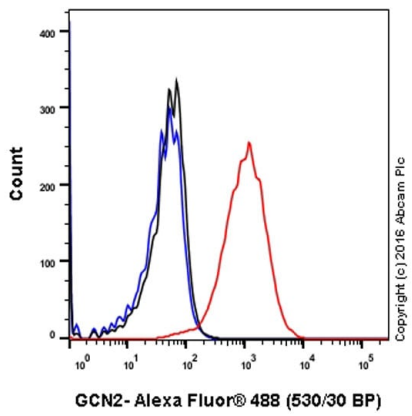
Immunocytochemistry/ Immunofluorescence - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

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.



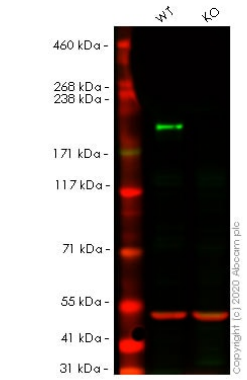
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

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 (Intracellular) - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

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.



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

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.

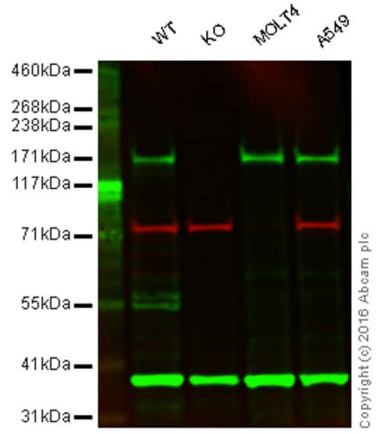
Performed under reducing conditions.

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

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 (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.

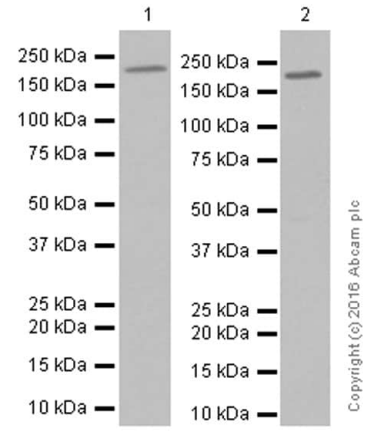


Western blot - Anti-GCN2 antibody [EPR5970(2)] (ab134053)

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.



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

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

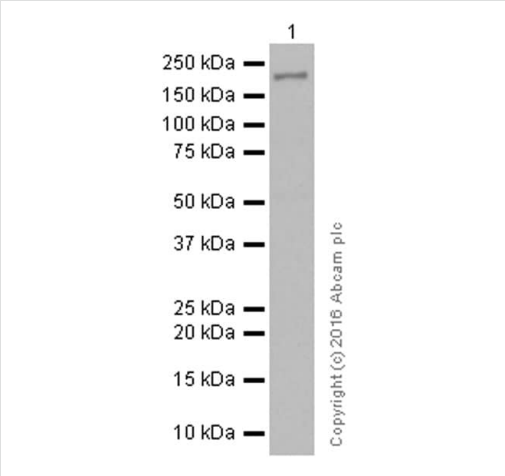
Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

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.



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

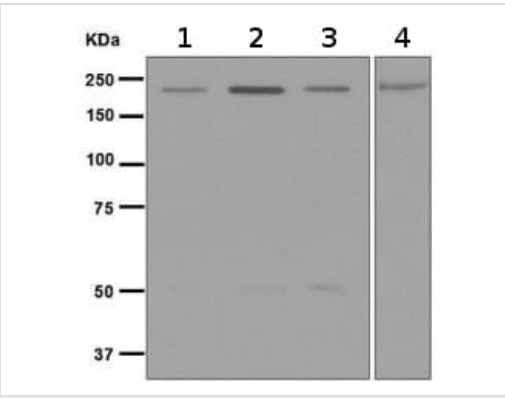
Secondary

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.



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

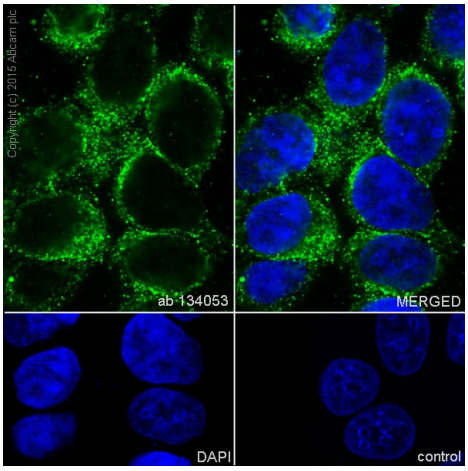
Lane 4 : A549 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

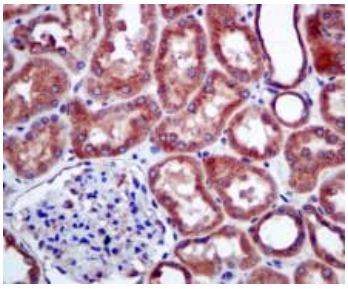
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 187 kDa



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

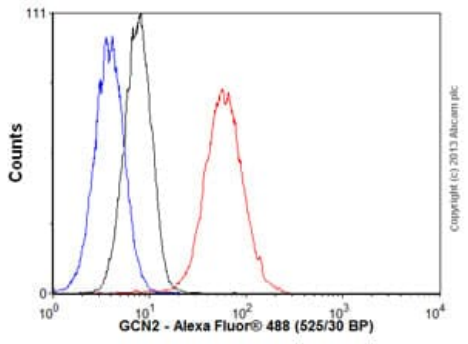
Immunofluorescence staining of MCF-7 cells with purified ab134053 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was an Alexa Fluor® 488 conjugated goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. The cells were fixed in 100% methanol. The negative control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



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

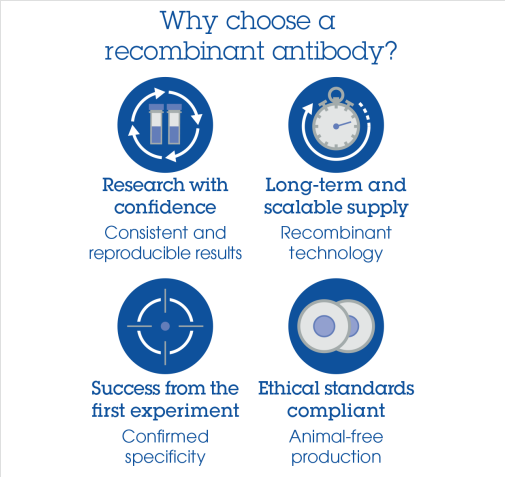
Immunohistochemical analysis of paraffin-embedded Human kidney tissue labelling GCN2 with unpurified ab134053 at 1/100 dilution.

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



Flow Cytometry (Intracellular) - 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.



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

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