abcam

ab32052

Anti-PKR antibody [YE350]

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	YE350
Purification technique	Affinity purification Protein A
Specificity	This antibody recognises Protein kinase R (PKR). It does not cross-react with other GCN2 family members.
Concentration	0.279 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	1/50

Notes	**For unpurified use at 1/100 - 1/500.**

IHC-P

Tested

Species	Human
Dilution info	1/100
Notes	-

IP

Tested

Species	Human
Dilution info	1/20 - 1/100
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000 - 1/10000
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20 - 1/50
Notes	-

Target data

See full target information EIF2AK2

Function	IFN-induced dsRNA-dependent serine/threonine-protein kinase that phosphorylates the alpha subunit of eukaryotic translation initiation factor 2 (EIF2S1/eIF-2-alpha) and plays a key role in the innate immune response to viral infection (PubMed:18835251, PubMed:19189853, PubMed:19507191, PubMed:21072047, PubMed:21123651, PubMed:22381929, PubMed:22948139, PubMed:23229543). Inhibits viral replication via the integrated stress response (ISR): EIF2S1/eIF-2-alpha phosphorylation in response to viral infection converts EIF2S1/eIF-2-alpha in a global protein synthesis inhibitor, resulting to a shutdown of cellular and viral protein synthesis, while concomitantly initiating the preferential translation of ISR-specific mRNAs, such as the transcriptional activator ATF4 (PubMed:19189853, PubMed:21123651, PubMed:22948139, PubMed:23229543). Exerts its antiviral activity on a wide range of DNA and RNA viruses including hepatitis C virus (HCV), hepatitis B virus (HBV), measles virus (MV) and herpes simplex virus 1 (HHV-1) (PubMed:11836380, PubMed:19189853, PubMed:19840259, PubMed:20171114, PubMed:21710204, PubMed:23115276, PubMed:23399035). Also involved in the regulation of signal transduction, apoptosis, cell proliferation and differentiation: phosphorylates other substrates including p53/TP53, PPP2R5A, DHX9, ILF3, IRS1 and the HHV-1 viral protein US11 (PubMed:11836380, PubMed:19229320, PubMed:22214662). In addition to serine/threonine-protein kinase activity, also has tyrosine-protein kinase activity and phosphorylates CDK1 at 'Tyr-4' upon DNA damage, facilitating its ubiquitination and proteasomal degradation (PubMed:20395957). Either as an adapter protein and/or via its kinase activity, can regulate various signaling pathways (p38 MAP kinase, NF-kappa-B and insulin signaling pathways) and transcription factors (JUN, STAT1, STAT3, IRF1, ATF3) involved in the expression of genes encoding pro-inflammatory cytokines and IFNs (PubMed:22948139, PubMed:23084476, PubMed:23372823). Activates the NF-kappa-B pathway via interaction with IKBKB and TRAF family of proteins and activates the p38 MAP kinase pathway via interaction with MAP2K6 (PubMed:10848580, PubMed:15121867, PubMed:15229216). Can act as both a positive and negative regulator of the insulin signaling pathway (ISP) (PubMed:20685959). Negatively regulates ISP by inducing the inhibitory phosphorylation of insulin receptor substrate 1 (IRS1) at 'Ser-312' and positively regulates ISP via phosphorylation of PPP2R5A which activates FOXO1, which in turn up-regulates the expression of insulin receptor substrate 2 (IRS2) (PubMed:20685959). Can regulate NLRP3 inflammasome assembly and the activation of NLRP3, NLRP1, AIM2 and NLRC4 inflammasomes (PubMed:22801494). Plays a role in the regulation of the cytoskeleton by binding to gelsolin (GSN), sequestering the protein in an inactive conformation away from actin (By similarity).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C

Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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.

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

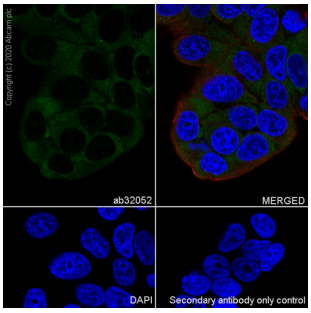
We do not recommend this combination. It is not covered by our product promise.

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.

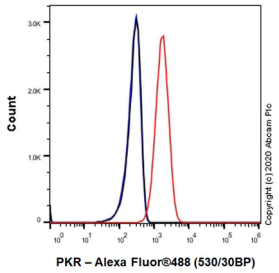
9 product images



Immunocytochemistry/ Immunofluorescence - Anti-PKR antibody [YE350] (ab32052)

PKR Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PKR antibody

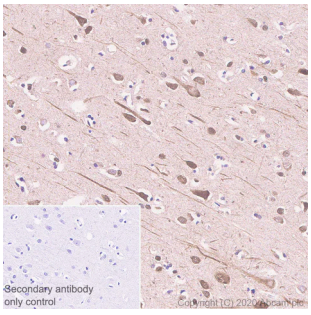
Immunocytochemistry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling PKR with Purified ab32052 at 1:50 dilution (5.04 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488,ab150077) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-PKR antibody [YE350] (ab32052)

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

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling PKR with Purified ab32052 at 1/30 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKR antibody [YE350] (ab32052)

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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cerebrum tissue sections labeling PKR with Purified ab32052 at 1:100 dilution (2.52 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

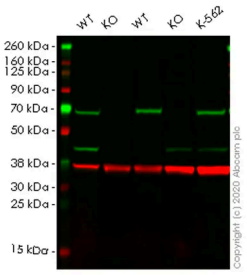


Immunoprecipitation - Anti-PKR antibody [YE350] (ab32052)

Purified ab32052 at 1/20 dilution (1µg) immunoprecipitating PKR in Jurkat whole cell lysate.
Lane 1 (input): Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10µg
Lane 2 (+): ab32052 + Jurkat whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32052 in Jurkat whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (ab131366) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 68 kDa
Possible degradation bands are observed between 20-30kDa.
All lanes:
Immunoprecipitation - Anti-PKR antibody [YE350] (ab32052)

Predicted band size: 62 kDa



Western blot - Anti-PKR antibody [YE350] (ab32052)

PKR Western blot staining using rabbit Anti-PKR antibody

Lanes 1-5: Merged signal (red and green). Green - ab32052 observed at 70 kDa. Red - loading control ab8245 observed at 36 kDa.
ab32052 Anti-PKR antibody [YE350] was shown to specifically react with PKR in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261824 (knockout cell lysate ab256899) was used. Wild-type and PKR knockout samples were subjected to SDS-PAGE. ab32052 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 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-PKR antibody [YE350] (ab32052) at 1/1000 dilution

Lane 1:
Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 2:
EIF2AK2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 2:
Western blot - Human EIF2AK2 (PKR) knockout HeLa cell lysate (ab256899) at 20 µg

Lane 3:
Wild-type A549 (Human lung carcinoma cell line) whole cell lysate at 20 µg

Lane 4:
EIF2AK2 knockout A549 (Human lung carcinoma cell line) whole cell lysate at 20 µg

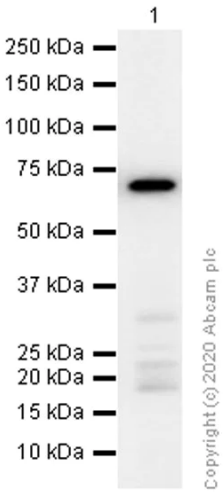
Lane 5:
K562 (Human chronic myelogenous leukemia lymphoblast cell line) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 62 kDa

Observed band size: 70 kDa



Western blot - Anti-PKR antibody [YE350] (ab32052)

Blocking buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-PKR antibody [YE350] (ab32052) at 1/5000 dilution

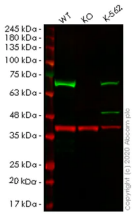
All lanes:
Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 62 kDa

Observed band size: 70 kDa



Western blot - Anti-PKR antibody [YE350] (ab32052)

PKR Western blot staining using rabbit Anti-PKR antibody

Lanes 1-3: Merged signal (red and green). Green - ab32052 observed at 70 kDa. Red - loading control ab8245 observed at 36 kDa.
ab32052 Anti-PKR antibody [YE350] was shown to specifically react with PKR in wild-type A549 cells. Loss of signal was observed when knockout cell line ab267000 (knockout cell lysate ab256901) was used. Wild-type and PKR knockout samples were subjected to SDS-PAGE. ab32052 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 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-PKR antibody [YE350] (ab32052) at 1/1000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
EIF2AK2 knockout A549 cell lysate at 20 µg

Lane 2:
Western blot - Human EIF2AK2 (PKR) knockout A549 cell line (ab267000)

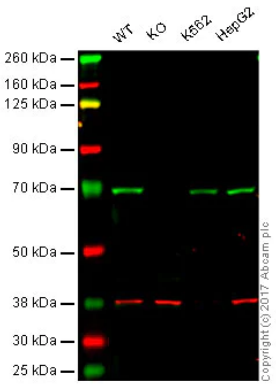
Lane 3:
K-562 cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 62 kDa

Observed band size: 70 kDa



Western blot - Anti-PKR antibody [YE350] (ab32052)

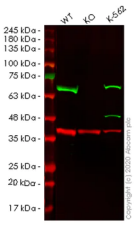
PKR Western blot staining using rabbit Anti-PKR antibody

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: EIF2AK2 knockout HAP1 whole cell lysate (20 µg)
Lane 3: K652 whole cell lysate (20 µg)
Lane 4: HepG2 whole cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab32052 observed at 70 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab32052 was shown to specifically react with EIF2AK2 when EIF2AK2 knockout samples were used. Wild-type and EIF2AK2 knockout samples were subjected to SDS-PAGE. ab32052 and ab9484 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-PKR antibody [YE350] (ab32052)

Predicted band size: 62 kDa



Western blot - Anti-PKR antibody [YE350] (ab32052)

PKR Western blot staining using rabbit Anti-PKR antibody

Lanes 1-3: Merged signal (red and green). Green - ab32052 observed at 70 kDa. Red - loading control ab8245 observed at 36 kDa.
ab32052 Anti-PKR antibody [YE350] was shown to specifically react with PKR in wild-type A549 cells. Loss of signal was observed when knockout cell line ab266999 (knockout cell lysate ab256900) was used. Wild-type and PKR knockout samples were subjected to SDS-PAGE. ab32052 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 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-PKR antibody [YE350] (ab32052) at 1/1000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
EIF2AK2 knockout A549 cell lysate at 20 µg

Lane 2:
Western blot - Human EIF2AK2 (PKR) knockout A549 cell line (ab266999)

Lane 3:
K-562 cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 62 kDa

Observed band size: 70 kDa

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