abcam

Product datasheet

Anti-PKR antibody [YE350] - BSA and Azide free ab239799

KO VALIDATED RabMAb

7 Images

Overview

Product name	Anti-PKR antibody [YE350] - BSA and Azide free
Description	Rabbit monoclonal [YE350] to PKR - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IP, ICC/IF, IHC-P, Flow Cyt, WB
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human PKR aa 50-150. The exact sequence is proprietary.
Positive control	WB: A549, K562, HAP1, HepG2, HeLa and MCF-7 cell lysates. ICC/IF: HeLa cells. IHC-P: Human stomach carcinoma and human liver tissues. Flow Cyt: MCF-7 cells.
General notes	ab239799 is the carrier-free version of ab32052 This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. 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. Ab239799 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* 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. Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough. Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility. We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in

our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	YE350
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab239799** in the following tested applications.

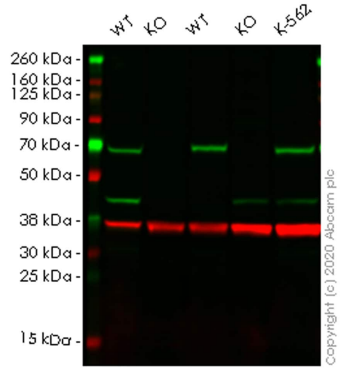
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 68 kDa (predicted molecular weight: 62 kDa).

Target

Function	Following activation by double-stranded RNA in the presence of ATP, the kinase becomes autophosphorylated and can catalyze the phosphorylation of the translation initiation factor EIF2S1, which leads to an inhibition of the initiation of protein synthesis. Double-stranded RNA is generated during the course of a viral infection.
Sequence similarities	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. GCN2 subfamily. Contains 2 DRBM (double-stranded RNA-binding) domains. Contains 1 protein kinase domain.
Post-translational modifications	Autophosphorylated on several Ser and Thr residues. Autophosphorylation of Thr-451 is dependent on Thr-446 and is stimulated by dsRNA binding and dimerization. Autophosphorylation apparently leads to the activation of the kinase.

Images



Western blot - Anti-PKR antibody [YE350] - BSA and Azide free (ab239799)

All lanes : Anti-PKR antibody [YE350] (ab32052) at 1/1000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : EIF2AK2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 4 : EIF2AK2 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 5 : K562 (Human chronic myelogenous leukemia lymphoblast cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

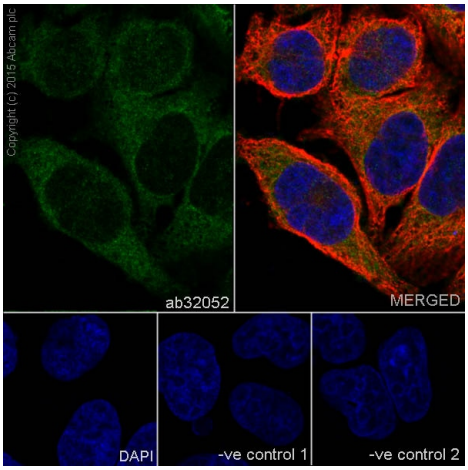
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 62 kDa
Observed band size: 70 kDa
why is the actual band size different from the predicted?

This data was developed using ab32052, the same antibody clone in a different buffer formulation.

Lanes 1-5: Merged signal (red and green). Green - ab32052 observed at 70 kDa. Red - loading control ab8245 observed at 36 kDa.

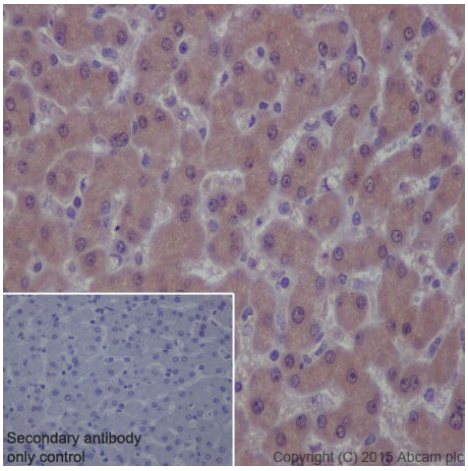
ab32052 Anti-PKR antibody [YE350] was shown to specifically react with PKR in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261824 (knockout cell lysate ab256899) was used. Wild-type and PKR knockout samples were subjected to SDS-PAGE. ab32052 and Anti-GAPDH antibody [6C5] - 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.



Immunocytochemistry/ Immunofluorescence - Anti-PKR antibody [YE350] - BSA and Azide free (ab239799)

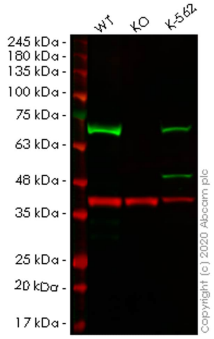
Immunofluorescence staining of HeLa cells with purified ab32052 at a working dilution of 1/100, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab32052 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32052).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKR antibody [YE350] - BSA and Azide free (ab239799)

Immunohistochemical staining of paraffin embedded human liver with purified ab32052 at a working dilution of 1/100. The secondary antibody used is ab97051, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32052).



Western blot - Anti-PKR antibody [YE350] - BSA and Azide free (ab239799)

All lanes : Anti-PKR antibody [YE350] (ab32052) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : EIF2AK2 knockout A549 cell lysate
Lane 3 : K-562 cell lysate

Lysates/proteins at 20 µg per lane.

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

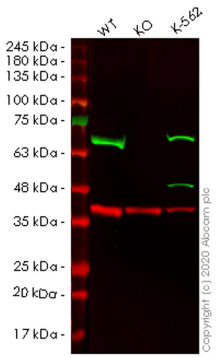
Predicted band size: 62 kDa
Observed band size: 70 kDa why is the actual band size different from the predicted?

This data was developed using the same antibody clone in a different buffer formulation (ab32052).

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.



Western blot - Anti-PKR antibody [YE350] - BSA and Azide free (ab239799)

All lanes : Anti-PKR antibody [YE350] (ab32052) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : EIF2AK2 knockout A549 cell lysate
Lane 3 : K-562 cell lysate

Lysates/proteins at 20 µg per lane.

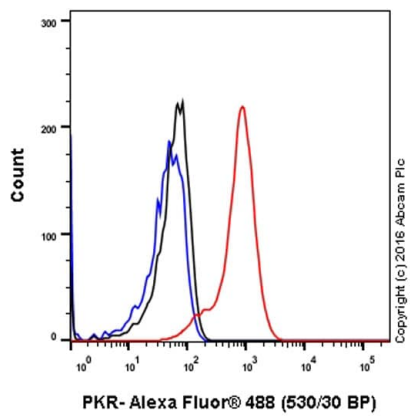
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 62 kDa
Observed band size: 70 kDa why is the actual band size different from the predicted?

This data was developed using the same antibody clone in a different buffer formulation (ab32052).

Lanes 1-3: Merged signal (red and green). Green - ab32052 observed at 70 kDa. Red - loading control ab8245 observed at 36 kDa.

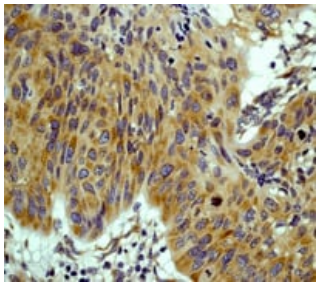
ab32052 Anti-PKR antibody [YE350] was shown to specifically react with PKR in wild-type A549 cells. Loss of signal was observed when knockout cell line ab266999 (knockout cell lysate ab256900) was used. Wild-type and PKR knockout samples were subjected to SDS-PAGE. ab32052 and Anti-GAPDH antibody [6C5] - 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.



Flow Cytometry - Anti-PKR antibody [YE350] - BSA and Azide free (ab239799)

Flow Cytometry analysis of MCF-7 cells labelling PKR with purified ab32052 at a dilution of 1/20 (red). Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. An Alexa Flour® 488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32052).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKR antibody [YE350] - BSA and Azide free (ab239799)

Immunohistochemical analysis of paraffin-embedded human stomach carcinoma using unpurified ab32052 at 1/250 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32052).

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