abcam

Product datasheet

Anti-PKR antibody [YE350] ab32052

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-PKR antibody [YE350]
Description	Rabbit monoclonal [YE350] to PKR
Host species	Rabbit
Specificity	This antibody recognises Protein kinase R (PKR). It does not cross-react with other GCN2 family members.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, IP, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human PKR aa 50-150. The exact sequence is proprietary. Database link: **P19525**
Positive control	WB: Jurkat, A549, K562, HAP1, HepG2, and HeLa cell lysates. IP: Jurkat cell lysat; IHC: Human cerebrum tissue; ICC/IF: MCF7 cells; Flow Cyt (intra): HeLa cells.
General notes	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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	YE350
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32052 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
Flow Cyt (Intra)		1/20 - 1/50.
WB	★★★★★ **(1)**	1/1000 - 1/10000. Detects a band of approximately 68 kDa (predicted molecular weight: 62 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
IP		1/20 - 1/100.
ICC/IF		1/50. **For unpurified use at 1/100 - 1/500.**

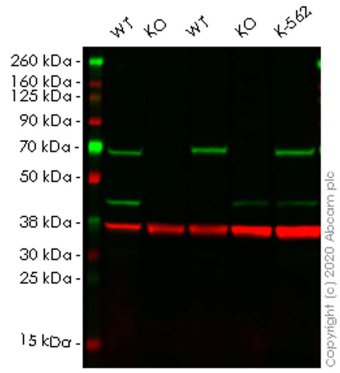
Target

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] (ab32052)

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

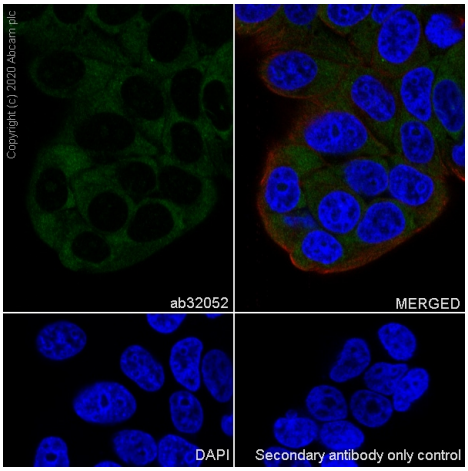
Lysates/proteins at 20 µg per lane.

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

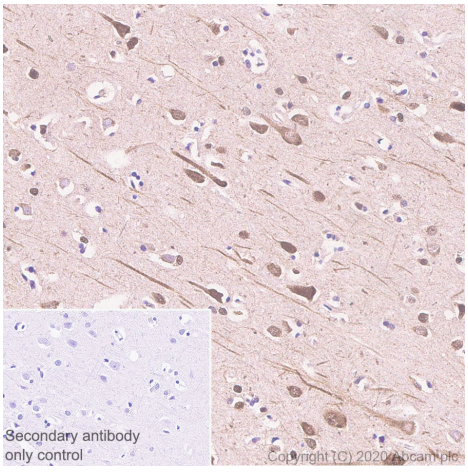
Lanes 1-5: Merged signal (red and green). Green - ab32052 observed at 70 kDa. Red - loading control **ab8245** observed at 36 kDa.

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.



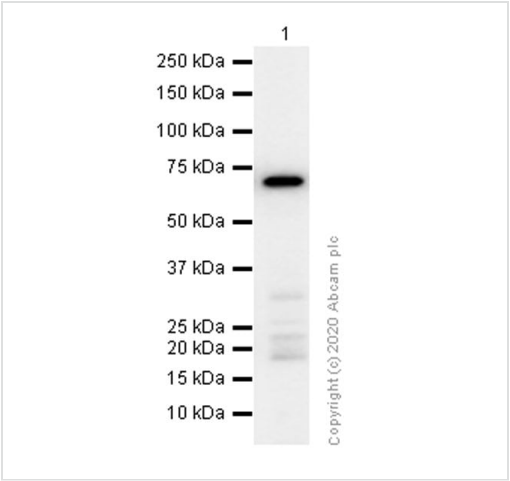
Immunocytochemistry/ Immunofluorescence - Anti-PKR antibody [YE350] (ab32052)

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.



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

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.



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

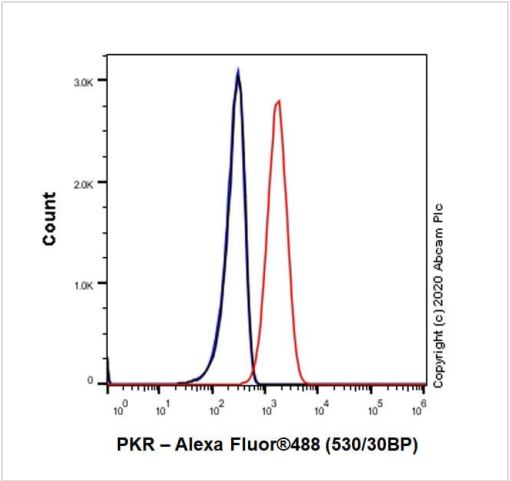
Anti-PKR antibody [YE350] (ab32052) at 1/5000 dilution (Purified) + Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

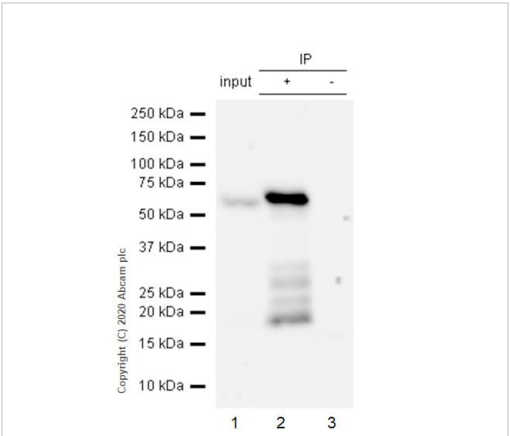
Predicted band size: 62 kDa
Observed band size: 70 kDa

Blocking buffer: 5% NFDM/TBST



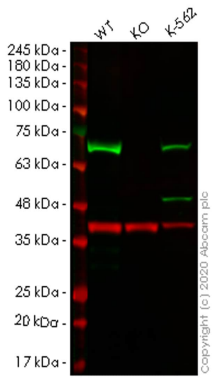
Flow Cytometry (Intracellular) - Anti-PKR antibody [YE350] (ab32052)

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).



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.



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

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

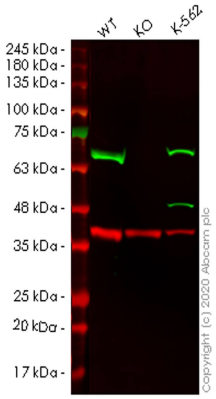
Lysates/proteins at 20 µg per lane.

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

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] (ab32052)

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

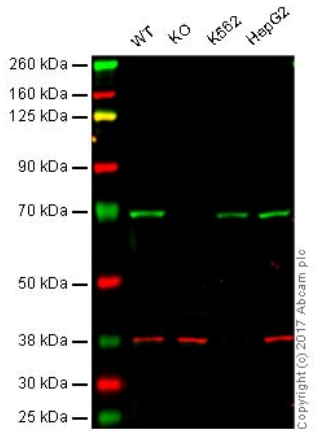
Lysates/proteins at 20 µg per lane.

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

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.



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

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.

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