abcam

Product datasheet

Anti-PKR antibody [EPR19374] ab184257

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 10 References 12 Images

Overview

Product name	Anti-PKR antibody [EPR19374]
Description	Rabbit monoclonal [EPR19374] to PKR
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, IP, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A459, K562, HeLa, Jurkat, 4T1, MCF7, HepG2 and bEnd.3 whole cell lysates. Mouse brain, cerebral cortex, hippocampus, lung, thymus and heart lysates; Rat brain, cerebral cortex, heart and spleen lysates; ICC/IF: bEnd.3 and A459 cells. Flow Cyt (Intra): bEnd.3 cells. IP: Mouse hippocampus lysate.
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**.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19374

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab184257 in the following tested applications.

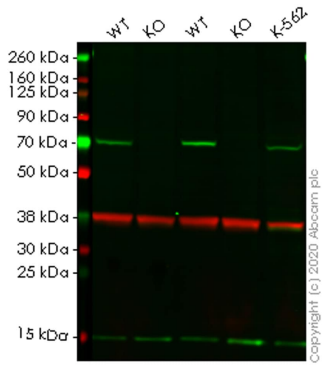
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/120.
ICC/IF		1/100.
IP		1/40.
WB	★★★★★ (1)	1/2000. Detects a band of approximately 58 kDa (predicted molecular weight: 58 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 [EPR19374] (ab184257)

All lanes : Anti-PKR antibody [EPR19374] (ab184257) 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

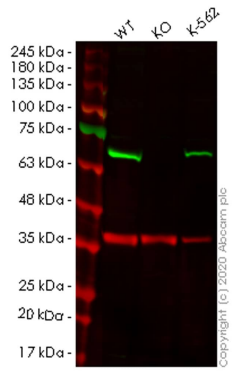
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 58 kDa
Observed band size: 70 kDa

Lanes 1-5: Merged signal (red and green). Green - ab184257 observed at 70 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab184257 Anti-PKR antibody [EPR19374] 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. ab184257 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.



Western blot - Anti-PKR antibody [EPR19374] (ab184257)

All lanes : Anti-PKR antibody [EPR19374] (ab184257) at 1/1000 dilution

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

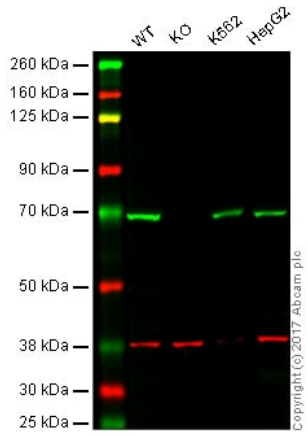
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 58 kDa
Observed band size: 70 kDa

Lanes 1-3: Merged signal (red and green). Green - ab184257 observed at 70 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab184257 Anti-PKR antibody [EPR19374] 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. ab184257 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.

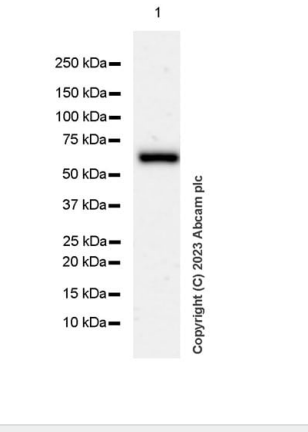


Western blot - Anti-PKR antibody [EPR19374] (ab184257)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: EIF2AK2 knockout HAP1 whole cell lysate (20 µg)
Lane 3: K562 whole cell lysate (20 µg)
Lane 4: HepG2 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab184257 observed at 70 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab184257 was shown to specifically react with EIF2AK2 when EIF2AK2 knockout samples were used. Wild-type and EIF2AK2 knockout samples were subjected to SDS-PAGE. Ab184257 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.



Western blot - Anti-PKR antibody [EPR19374] (ab184257)

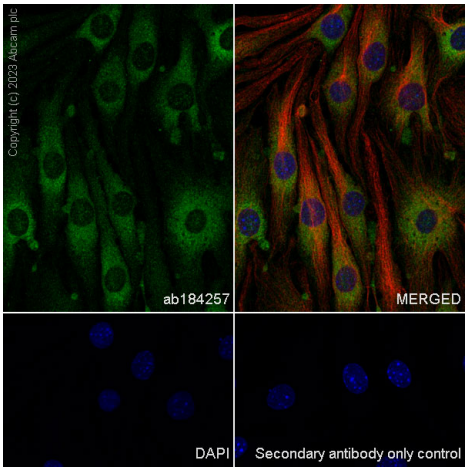
Anti-PKR antibody [EPR19374] (ab184257) at 1/1000 dilution + bEnd.3 (mouse brain endothelial cell) whole cell lysate at 20 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 58 kDa

Exposure time: 114 seconds

Blocking and diluting buffer: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-PKR antibody [EPR19374] (ab184257)

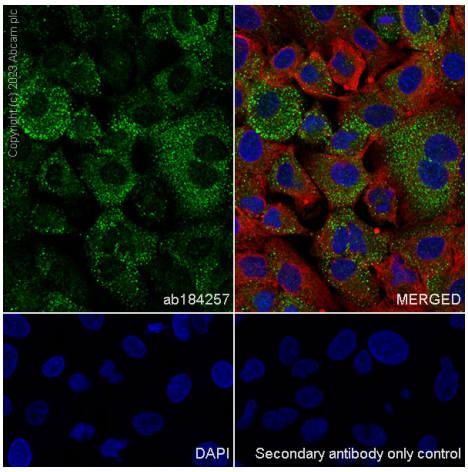
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized bEnd.3 (mouse brain endothelial cell) labeling PKR with ab184257 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preabsorbed (**ab150081**) secondary antibody at 1/1000 dilution (green).

Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 was used as a counterstain

The nuclear counterstain is DAPI (blue)

Confocal image showing cytoplasmic and weak nuclear staining in bEnd.3 cell line.

Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-PKR antibody [EPR19374] (ab184257)

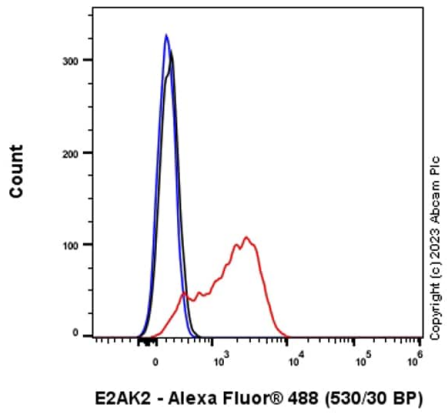
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A549 (human lung carcinoma epithelial cell) labeling PKR with ab184257 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preabsorbed (**ab150081**) secondary antibody at 1/1000 dilution (green).

Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 was used as a counterstain

The nuclear counterstain is DAPI (blue)

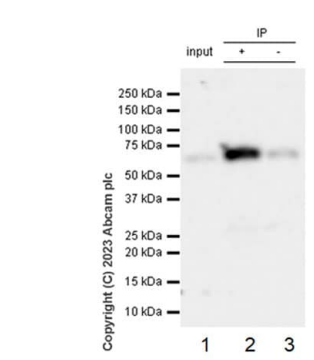
Confocal image showing cytoplasmic and weak nuclear staining in A549 cell line.

Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8).



Flow Cytometry (Intracellular) - Anti-PKR antibody [EPR19374] (ab184257)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilised bEnd.3 (mouse brain endothelial cell) cells labeling PKR with ab184257 at 1/500 dilution (red) compared with a Rabbit monoclonal IgG isotype control (**ab172730**; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/5000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-PKR antibody [EPR19374] (ab184257)

PKR was immunoprecipitated from mouse hippocampus lysate with ab184257 at 1/30 dilution (2µg in 0.35mg lysates).

Western blot was performed from the immunoprecipitate using ab184257 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/5000 dilution.

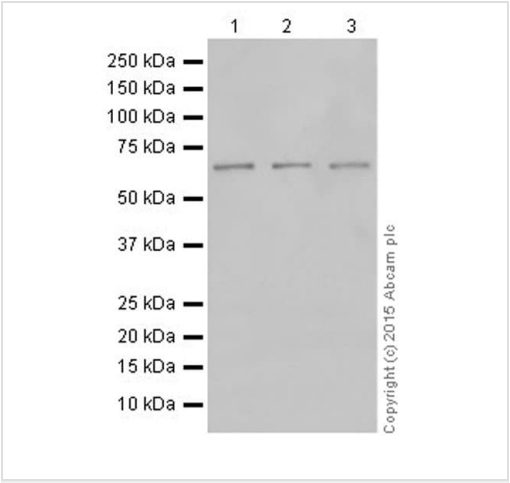
Lane 1: Mouse hippocampus tissue lysate 10 µg (Input).

Lane 2: ab184257 IP in Mouse hippocampus tissue lysate.

Lane 3: Rabbit IgG,monoclonal [EPR19374] - Isotype Control (**ab172730**) instead of ab184257 in mouse hippocampus tissue lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 31 seconds.



Western blot - Anti-PKR antibody [EPR19374] (ab184257)

All lanes : Anti-PKR antibody [EPR19374] (ab184257) at 1/2000 dilution

Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

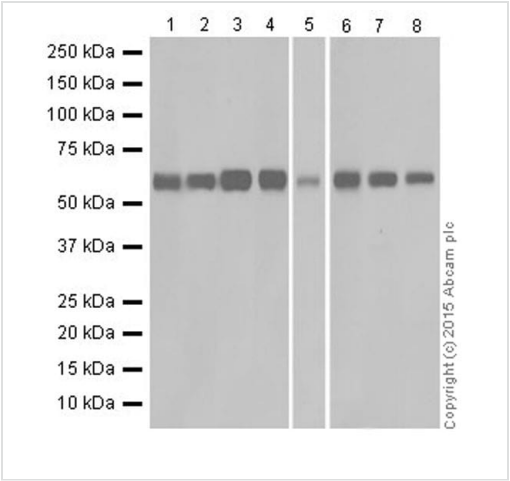
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 58 kDa
Observed band size: 58 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PKR antibody [EPR19374] (ab184257)

All lanes : Anti-PKR antibody [EPR19374] (ab184257) at 1/2000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Mouse cerebral cortex lysate
Lane 3 : Mouse hippocampus lysate
Lane 4 : Mouse lung lysate
Lane 5 : Mouse thymus lysate
Lane 6 : Rat brain lysate
Lane 7 : Rat cerebral cortex lysate
Lane 8 : 4T1 (Mouse mammary gland carcinoma cell line) whole cell lysate

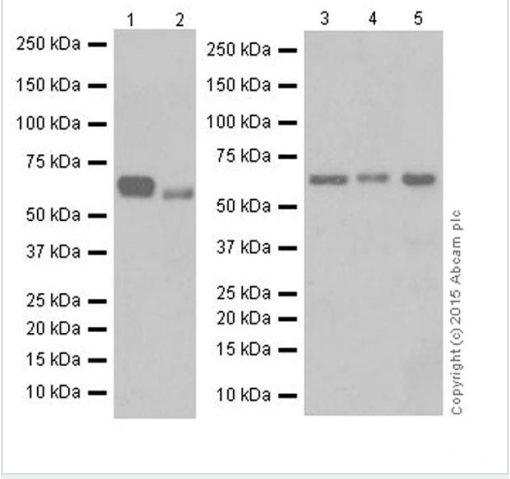
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 58 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1,2,3 and 4: 3 minutes; Lane 5: 30 seconds; Lane 6,7 and 8: 10 seconds.



Western blot - Anti-PKR antibody [EPR19374] (ab184257)

All lanes : Anti-PKR antibody [EPR19374] (ab184257) at 1/2000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Mouse heart lysate
Lane 3 : Rat brain lysate
Lane 4 : Rat heart lysate
Lane 5 : Rat spleen lysate

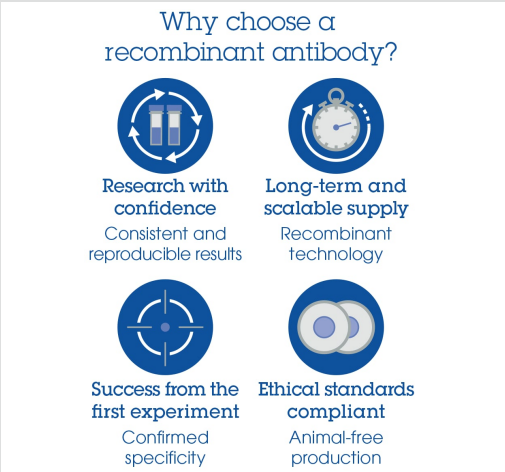
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 58 kDa
Observed band size: 58 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 2: 3 minutes; Lane 3,4 and 5: 5 seconds.



Anti-PKR antibody [EPR19374] (ab184257)

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