abcam

Product datasheet

Anti-PKR antibody [EPR19374] - BSA and Azide free ab224887

KO VALIDATED Recombinant RabMAb

8 Images

Overview

Product name	Anti-PKR antibody [EPR19374] - BSA and Azide free
Description	Rabbit monoclonal [EPR19374] to PKR - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF, IP
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	ab224887 is the carrier-free version of **ab184257**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. 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. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19374
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab224887 in the following tested applications.

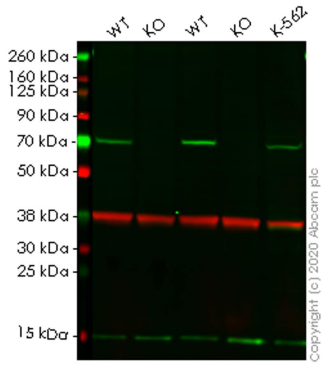
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 58 kDa (predicted molecular weight: 58 kDa).
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

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] - BSA and Azide free (ab224887)

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.

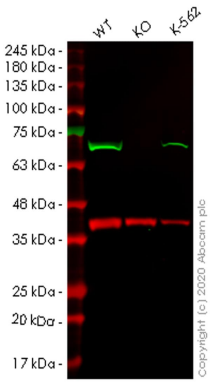
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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

This data was developed using **ab184257**, the same antibody clone in a different buffer formulation.

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] - BSA and Azide free (ab224887)

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.

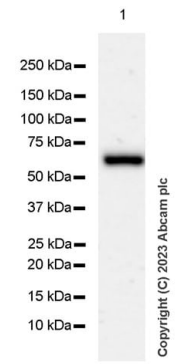
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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

This data was developed using the same antibody clone in a different buffer formulation (**ab184257**).

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 **ab266999** (knockout cell lysate **ab256900**) 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] - BSA and Azide free (ab224887)

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

Secondary

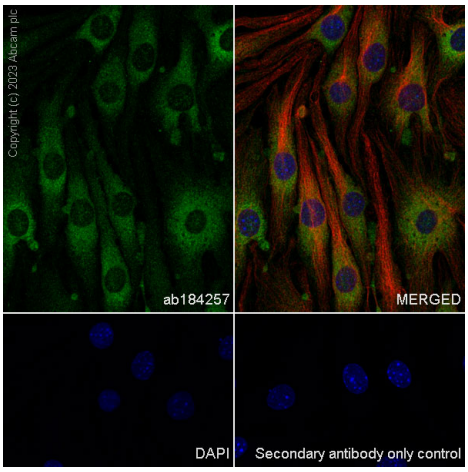
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 58 kDa

Exposure time: 114 seconds

This data was developed using **ab184257**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-PKR antibody [EPR19374] - BSA and Azide free (ab224887)

This data was developed using **ab184257**, the same antibody clone in a different buffer formulation.

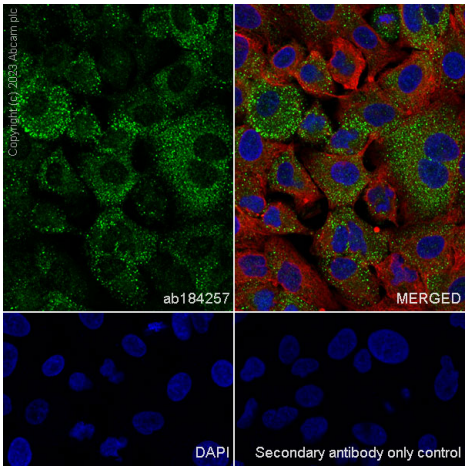
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized bEnd.3 (mouse brain endothelial cell) labeling PKR with **ab184257** at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preabsorbed (**ab150081**) secondary antibody at 1/1000 dilution (green).

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] - BSA and Azide free (ab224887)

This data was developed using **ab184257**, the same antibody clone in a different buffer formulation.

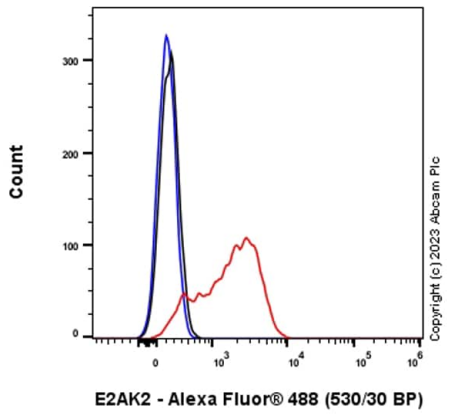
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A549 (human lung carcinoma epithelial cell) labeling PKR with **ab184257** at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preabsorbed (**ab150081**) secondary antibody at 1/1000 dilution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

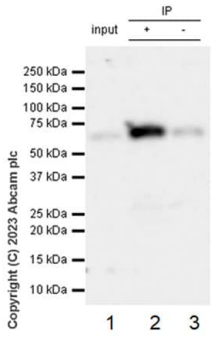
Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8).



Flow Cytometry (Intracellular) - Anti-PKR antibody [EPR19374] - BSA and Azide free (ab224887)

This data was developed using **ab184257**, the same antibody clone in a different buffer formulation.

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] - BSA and Azide free (ab224887)

This data was developed using **ab184257**, the same antibody clone in a different buffer formulation.

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.

Anti-PKR antibody [EPR19374] - BSA and Azide free (ab224887)

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