abcam

Product datasheet

Anti-PKR antibody [Y117] ab32506

KO VALIDATED Recombinant RabMAb

3 Abreviews 25 References 11 Images

Overview

Product name	Anti-PKR antibody [Y117]
Description	Rabbit monoclonal [Y117] to PKR
Host species	Rabbit
Specificity	This antibody does not cross-react with other GCN2 family members.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human PKR aa 500-600 (C terminal). The exact sequence is proprietary.
Positive control	WB: MCF-7, HEK293 and HeLa whole cell lysate (**ab150035**) and human liver carcinoma tissue lysate. ICC/IF: Wild type HAP1, MCF-7 and HeLa cells. IHC-P: Human liver carcinoma and colon carcinoma tissue.
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	Y117
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32506 in the following tested applications.

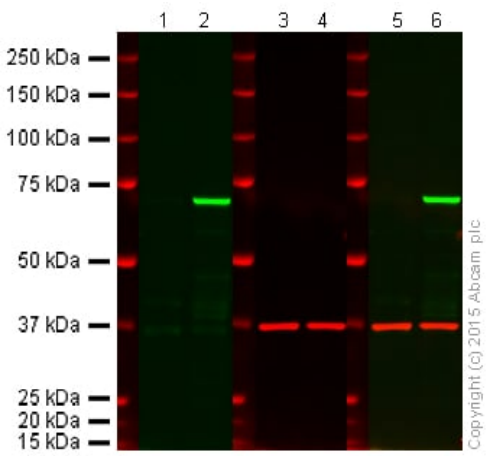
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB	★★★★☆ **(3)**	1/5000 - 1/20000. 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.
ICC/IF		1/100. **For unpurified use at 1/250 - 1/500**
IP		1/80 - 1/100.

Target

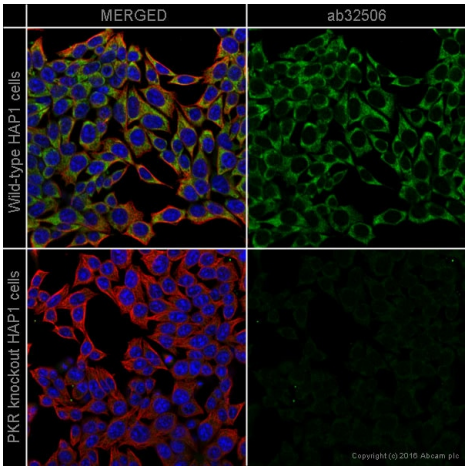
Function	Following activation by double-stranded RNA in the presence of ATP, the kinase becomes autophosphorylated and can catalyze the phosphorylation of the translation initiation factor EIF2S1, which leads to an inhibition of the initiation of protein synthesis. Double-stranded RNA is generated during 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 [Y117] (ab32506)

Lanes 1, 3 and 5: PKR knockout HAP1 cell lysate (20 µg)
Lanes 2, 4 and 6: Wild-type HAP1 cell lysate (20 µg)
Lanes 1 and 2: Green signal from target - ab32506 observed at 62 kDa
Lanes 3 and 4: Red signal from loading control - **ab8245** observed at 37 kDa
Lanes 5 and 6: Merged (red and green) signal
ab32506 was shown to specifically react with PKR when PKR knockout samples were used. Wild-type and PKR knockout samples were subjected to SDS-PAGE. ab32506 and **ab8245** (loading control to GAPDH) were diluted 1/10 000 and 1/2000 respectively and incubated overnight at 4°C. 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 1/10 000 dilution for 1 h at room temperature before imaging.

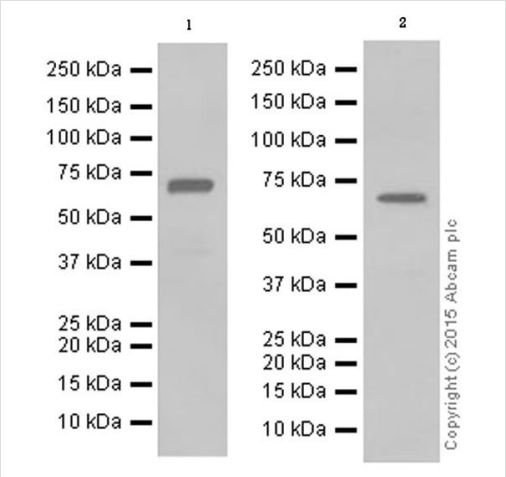


Immunocytochemistry/ Immunofluorescence - Anti-PKR antibody [Y117] (ab32506)

ab32506 staining PKR in wild-type HAP1 cells (top panel) and PKR knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab32506 at 1/400 dilution and **ab7291** at 1ug/ml concentration overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to Mouse IgG (Alexa Fluor® 594) (**ab150117**) at 2ug/ml (shown in pseudo-color red). Nuclear DNA was labelled in blue with DAPI.

This product also gave a positive signal under the same testing conditions in HAP1 cells fixed with 4% formaldehyde (10 min).

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



Western blot - Anti-PKR antibody [Y117] (ab32506)

All lanes : Anti-PKR antibody [Y117] (ab32506) at 1/20000 dilution

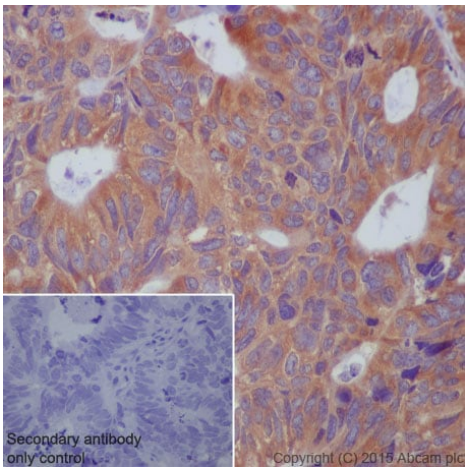
Lane 1 : MCF-7 (human breast carcinoma) whole cell lysates
Lane 2 : HEK293 (human embryonic kidney) whole cell lysates

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: 62 kDa
Additional bands at: 68 kDa. We are unsure as to the identity of these extra bands.

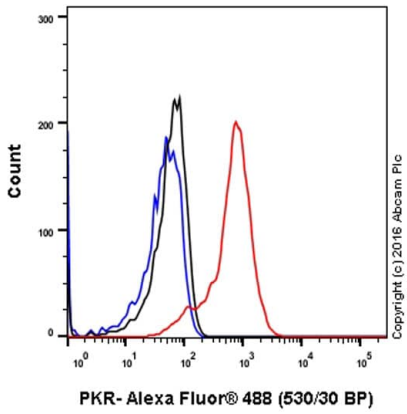
Purified format.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKR antibody [Y117] (ab32506)

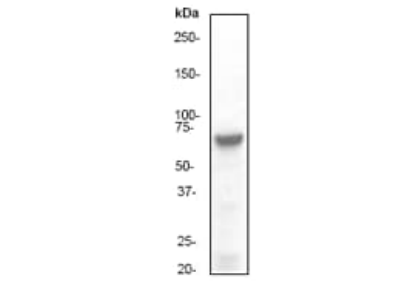
ab32506 staining PKR in human liver carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti-rabbit IgG H&L (HRP) **ab97051** was used as the secondary antibody at a dilution of 1/500.

Negative control 1: PBS in place of primary antibody.



Flow Cytometry (Intracellular) - Anti-PKR antibody [Y117] (ab32506)

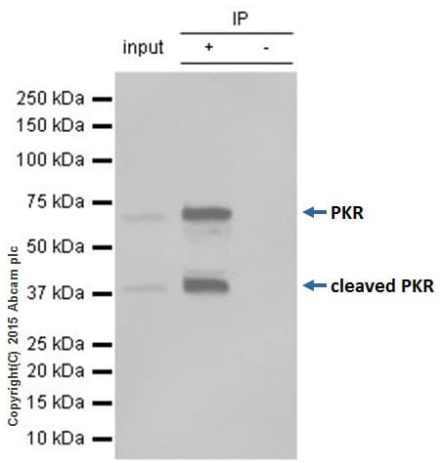
Intracellular Flow Cytometry analysis of MCF-7 (human breast carcinoma) cells labeling PKR with purified ab32506 at 1/20 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Western blot - Anti-PKR antibody [Y117] (ab32506)

Anti-PKR antibody [Y117] (ab32506) at 1/10000 dilution (unpurified) + MCF-7 cell lysate

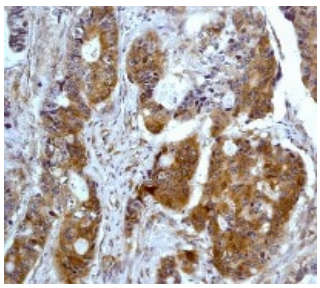
Predicted band size: 62 kDa
Observed band size: 68 kDa



Immunoprecipitation - Anti-PKR antibody [Y117] (ab32506)

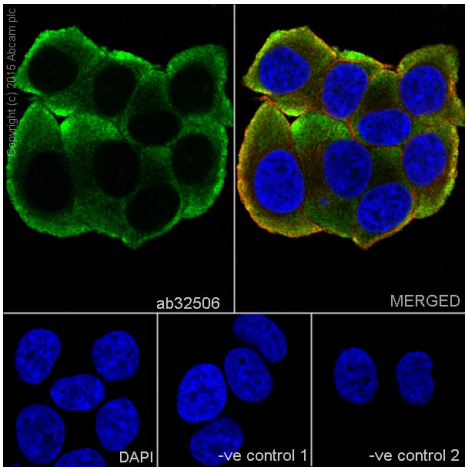
ab32506 immunoprecipitating PKR. 10µg of cell lysate was incubated with primary antibody at a dilution of 1/40 and VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at a dilution of 1/10000.

Lane 1: HEK293 (human embryonic kidney) whole cell lysate (10ug)
Lane 2: HEK293 (human embryonic kidney) whole cell lysate
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab32506 in HEK293 (human embryonic kidney) whole cell lysate



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKR antibody [Y117] (ab32506)

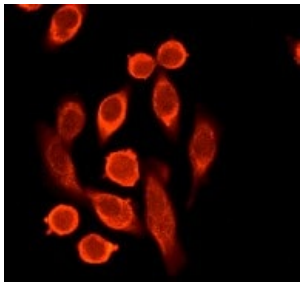
Immunohistochemical analysis of paraffin-embedded human colon carcinoma using unpurified ab32506 at 1/100 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-PKR antibody [Y117] (ab32506)

ab32506 staining PKR in MCF-7 (human breast carcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti rabbit IgG (Alexa Fluor® 488) (**ab150077**) was used as the secondary antibody. **ab7291** and **ab150120** were used as counterstains for primary antibody ab32506 and secondary antibody **ab150077** respectively and DAPI was used as a nuclear counterstain.

Negative control 1: Rabbit primary antibody and anti-mouse secondary antibody (**ab150120**)
Negative control 2: Mouse primary antibody (**ab7291**) and anti-rabbit secondary antibody (**ab150077**)



Immunocytochemistry/ Immunofluorescence - Anti-PKR antibody [Y117] (ab32506)

Immunofluorescent staining of HeLa cells using unpurified ab32506 at 1/250 dilution.

Anti-PKR antibody [Y117] (ab32506)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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