abcam

Product datasheet

Anti-PKR antibody [Y117] ab32506





重组 RabMAb

★★★★★ 3 Abreviews 25 References 11 图像

概述

产品名称 Anti-PKR抗体[Y117]

描述 兔单克隆抗体[Y117] to PKR

宿主 Rabbit

特异性 This antibody does not cross-react with other GCN2 family members.

经测试应用 适用于: Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP

种属反应性 与反应: Human

免疫原 Synthetic peptide within Human PKR aa 500-600 (C terminal). The exact sequence is proprietary.

阳性对照 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.

常规说明 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.

性能

形式

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯**度** Protein A purified

 克隆
 单克隆

 克隆编号
 Y117

 同种型
 IgG

应用

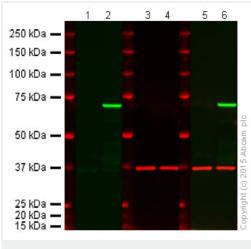
The Abpromise guarantee Abpromise™承诺保证使用ab32506于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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.

靶标	
功能	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.
序列相似性	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.
翻译后修饰	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.

图片



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

Immunocytochemistry/ Immunofluorescence - 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

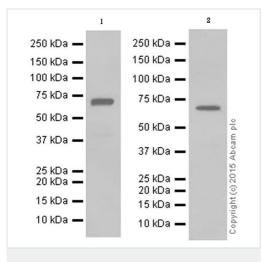
Lanes 3 and 4: Red signal from loading control - ab8245 observed at 37 kDa

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.

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 lgG (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

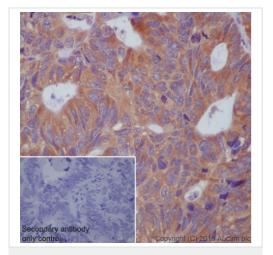
All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Predicted band size: 62 kDa

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

these extra bands.

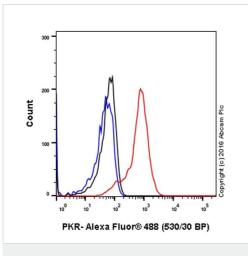




Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKR antibody [Y117] (ab32506)

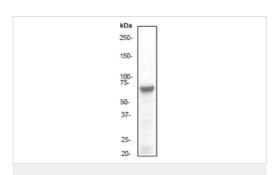
ab32506 staining PKR in human liver carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution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

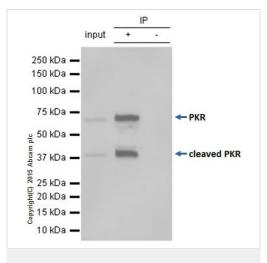
Intracellular Flow Cytometry analysis of MCF-7 (human breast carcinoma) cells labeling PKR with purified ab32506 at 1/20 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit lgG (Alexa Fluor[®] 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal lgG (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



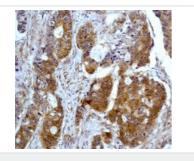
Immunoprecipitation - Anti-PKR antibody [Y117] (ab32506)

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)

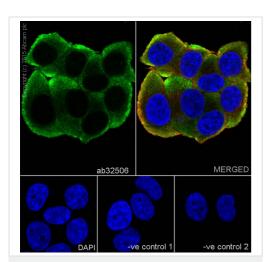
Lane 2: HEK293 (human embryonic kidney) whole cell lysate

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab32506 in HEK293 (human embryonic kidney) whole cell lysate



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

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

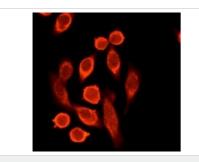


Immunocytochemistry/ Immunofluorescence - Anti-PKR antibody [Y117] (ab32506)

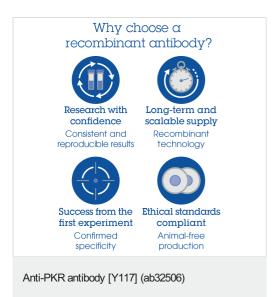
ab32506 staining PKR in MCF-7 (human breast carcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti rabbit IgG (Alexa Fluor® 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 (<u>ab150120</u>)

Negative control 2: Mouse primary antibody (<u>ab7291</u>) and antirabbit secondary antibody (<u>ab150077</u>)



Immunocytochemistry/ Immunofluorescence - Anti-PKR antibody [Y117] (ab32506) Immunofluorescent staining of HeLa cells using unpurified ab32506 at 1/250 dilution.



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