abcam

Product datasheet

Anti-PACT (PKR activating protein) / PRKRA antibody [EPR3224] ab75749

KO VALIDATED Recombinant RabMAb

11 References 5 Images

Overview

Product name	Anti-PACT (PKR activating protein) / PRKRA antibody [EPR3224]
Description	Rabbit monoclonal [EPR3224] to PACT (PKR activating protein) / PRKRA
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P **Unsuitable for:** Flow Cyt,ICC/IF or IP
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide within Human PACT (PKR activating protein)/ PRKRA aa 300-400 (C terminal). The exact sequence is proprietary.
Positive control	WB: HEK-293T, K562, Jurkat and HepG2 whole cell lysate (**ab7900**). IHC-P: Human brain 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR3224
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab75749 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
WB		
IHC-P		

Application notes IHC-P: 1/100 - 1/250. Perform heat mediated antigen retrieval using 0.01M Sodium Citrate Buffer, pH 6.0 before commencing with IHC staining protocol.
WB: 1/1000 - 1/5000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).

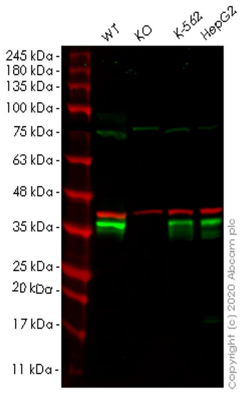
Is unsuitable for Flow Cyt, ICC or IP.

Not yet tested in other applications.
Optimal dilutions/concentrations should be determined by the end user.

Target

Function	Activates EIF2AK2/PKR in the absence of double stranded RNA (dsRNA), leading to phosphorylation of EIF2S1/EFI2-alpha and inhibition of translation and induction of apoptosis. Required for siRNA production by DICER1 and for subsequent siRNA-mediated post-transcriptional gene silencing. Does not seem to be required for processing of pre-miRNA to miRNA by DICER1.
Involvement in disease	Defects in PRKRA are the cause of dystonia type 16 (DYT16) [MIM:612067]. DYT16 is an early-onset dystonia-parkinsonism disorder. Dystonia is defined by the presence of sustained involuntary muscle contraction, often leading to abnormal postures. DYT16 patients have progressive, generalized dystonia with axial muscle involvement, oro-mandibular (sardonic smile) and laryngeal dystonia and, in some cases, parkinsonian features.
Sequence similarities	Belongs to the PRKRA family. Contains 3 DRBM (double-stranded RNA-binding) domains.
Domain	Self-association may occur via interactions between DRBM domains as follows: DRBM 1/DRBM 1, DRBM 1/DRBM 2, DRBM 2/DRBM 2 or DRBM 3/DRBM3.
Post-translational modifications	Phosphorylated at Ser-246 in unstressed cells and at Ser-287 in stressed cells. Phosphorylation at Ser-246 appears to be a prerequisite for subsequent phosphorylation at Ser-287. Phosphorylation at Ser-246 and Ser-287 are necessary for activation of EIF2AK2/PKR under conditions of stress.
Cellular localization	Cytoplasm > perinuclear region.

Images



Western blot - Anti-PACT (PKR activating protein) / PRKRA antibody [EPR3224] (ab75749)

All lanes : Anti-PACT (PKR activating protein) / PRKRA antibody [EPR3224] (ab75749) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : PRKRA knockout HEK-293T cell lysate
Lane 3 : K-562 cell lysate
Lane 4 : HepG2 cell lysate

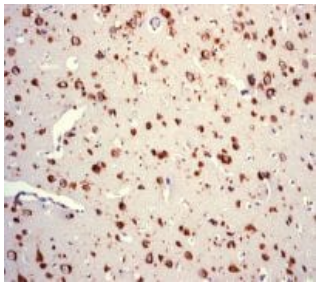
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 34 kDa
Observed band size: 36 kDa

Lanes 1-4: Merged signal (red and green). Green - ab75749 observed at 36 kDa. Red - loading control **ab8245** observed at 36 kDa.

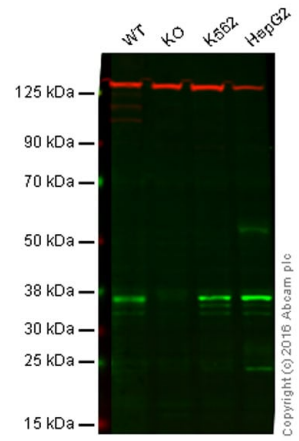
ab75749 Anti-PACT (PKR activating protein) / PRKRA antibody [EPR3224] was shown to specifically react with PACT in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line **ab266806** (knockout cell lysate **ab258141**) was used. Wild-type and PACT knockout samples were subjected to SDS-PAGE. ab75749 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PACT (PKR activating protein) / PRKRA antibody [EPR3224] (ab75749)

ab75749 at 1/100 dilution staining PACT (PKR activating protein) / PRKRA in paraffin-embedded human brain tissue.

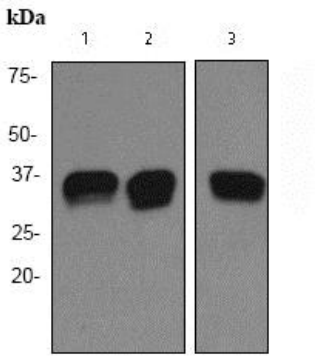
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-PACT (PKR activating protein) / PRKRA antibody [EPR3224] (ab75749)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: PACT (PKR activating protein)/PRKRA knockout HAP1 cell lysate (20 µg)
Lane 3: K562 cell lysate (20 µg)
Lane 4: HepG2 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab75749 observed at 36 kDa. Red - loading control, **ab18058**, observed at 124 kDa.

ab75749 was shown to specifically react with PACT (PKR activating protein)/PRKRA when PACT (PKR activating protein)/PRKRA knockout samples were used. Wild-type and PACT (PKR activating protein)/PRKRA knockout samples were subjected to SDS-PAGE. ab75749 and **ab18058** (loading control to Vinculin) were diluted at 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) **ab216776** secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-PACT (PKR activating protein) / PRKRA antibody [EPR3224] (ab75749)

All lanes : Anti-PACT (PKR activating protein) / PRKRA antibody [EPR3224] (ab75749) at 1/1000 dilution

Lane 1 : K562 cell lysate
Lane 2 : Jurkat cell lysate
Lane 3 : HepG2 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 34 kDa
Observed band size: 34 kDa

Anti-PACT (PKR activating protein) / PRKRA antibody [EPR3224] (ab75749)

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