abcam

Product datasheet

Anti-PACT (PKR activating protein) / PRKRA antibody ab31967

KO VALIDATED

4 References 6 Images

Overview

Product name	Anti-PACT (PKR activating protein) / PRKRA antibody
Description	Rabbit polyclonal to PACT (PKR activating protein) / PRKRA
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Cow
Immunogen	Synthetic peptide corresponding to Human PACT (PKR activating protein)/ PRKRA aa 100-200 conjugated to keyhole limpet haemocyanin. (Peptide available as **ab30768**)
Positive control	WB: HEK-293T, K562, HepG2, HAP1 and PC12 whole cell lysates; Mouse testis tissue lysate. ICC/IF: HeLa cells. IP: Mouse testis tissue.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS

	Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab31967 in the following tested applications.

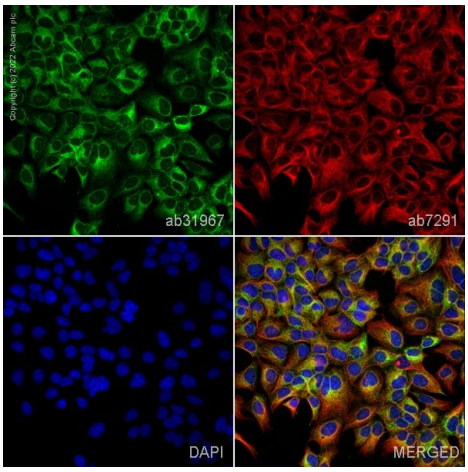
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use a concentration of 5 µg/ml.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).
IP		Use a concentration of 5 µg/ml.

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.

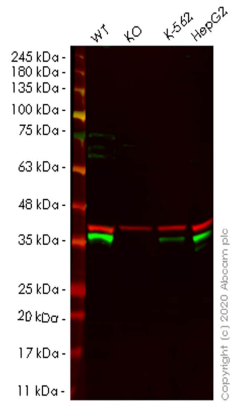
Images



Immunocytochemistry/ Immunofluorescence - Anti-PACT (PKR activating protein) / PRKRA antibody (ab31967)

ab31967 staining PACT (PKR activating protein) / PRKRA in Hek293 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-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 overnight at 4°C with ab31967 at 5µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Western blot - Anti-PACT (PKR activating protein) / PRKRA antibody (ab31967)

All lanes : Anti-PACT (PKR activating protein) / PRKRA antibody (ab31967) 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.

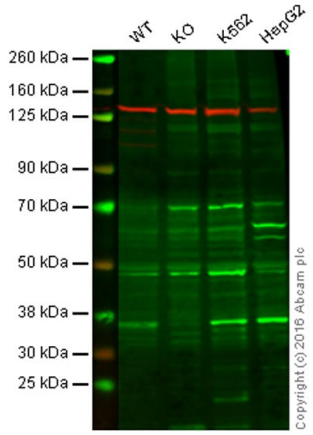
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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

Lanes 1-4: Merged signal (red and green). Green - ab31967 observed at 36 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab31967 Anti-PACT (PKR activating protein) / PRKRA antibody 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

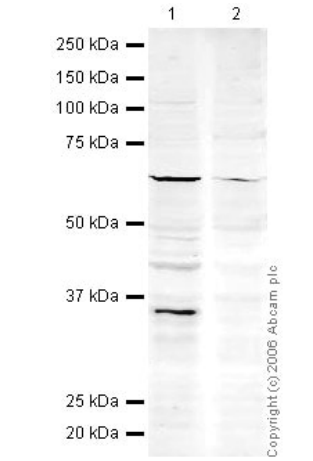
and PACT knockout samples were subjected to SDS-PAGE. ab31967 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**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-PACT (PKR activating protein) / PRKRA antibody (ab31967)

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 - ab31967 observed at 36 kDa. Red - loading control, **ab18058**, observed at 124 kDa.

ab31967 was shown to recognize PACT (PKR activating protein)/PRKRA when PACT (PKR activating protein)/PRKRA knockout samples were used, along with additional cross-reactive bands. Wild-type and PACT (PKR activating protein)/PRKRA knockout samples were subjected to SDS-PAGE. ab31967 and **ab18058** (loading control to Vinculin) were diluted at 1 µg/ml and 1/10000 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 1/10000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-PACT (PKR activating protein) / PRKRA antibody (ab31967)

All lanes : Anti-PACT (PKR activating protein) / PRKRA antibody (ab31967) at 1 µg/ml

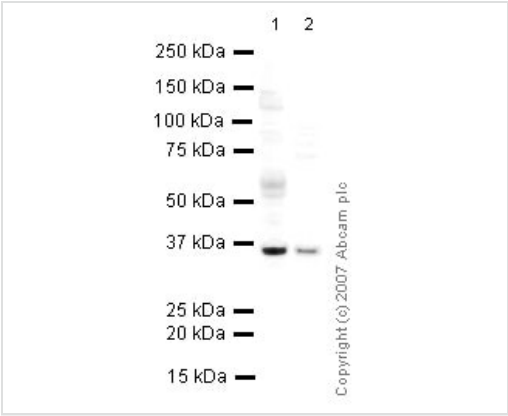
Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Human PACT (PKR activating protein) / PRKRA peptide (**ab30768**) at 1 µg/ml

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/15000 dilution

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 34 kDa
Additional bands at: 60 kDa (possible cross reactivity, but this band is not blocked)



Western blot - Anti-PACT (PKR activating protein) / PRKRA antibody (ab31967)

All lanes : Anti-PACT (PKR activating protein) / PRKRA antibody (ab31967) at 1 µg/ml

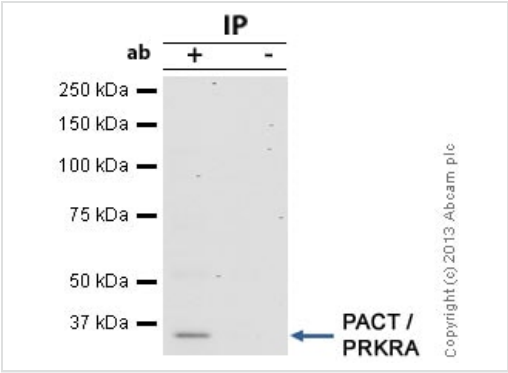
Lane 1 : Testis (Mouse) Tissue Lysate - normal tissue
Lane 2 : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 34 kDa
Additional bands at: 55 kDa. We are unsure as to the identity of these extra bands.



Immunoprecipitation - Anti-PACT (PKR activating protein) / PRKRA antibody (ab31967)

PACT (PKR activating protein) / PRKRA was immunoprecipitated using 0.5mg Mouse Testis tissue, 5µg of Rabbit polyclonal to PACT (PKR activating protein) / PRKRA and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Mouse Testis tissue lysate lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab31967.
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (**ab99697**).

Band: 34kDa; PACT (PKR activating protein) / PRKRA

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