


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

KO 評価済

4 References 画像数 6

製品の概要

| | |
|--------------|---|
| 製品名 | Anti-PACT (PKR activating protein) / PRKRA antibody |
| 製品の詳細 | Rabbit polyclonal to PACT (PKR activating protein) / PRKRA |
| 由来種 | Rabbit |
| アプリケーション | 適用あり: ICC/IF, WB, IP |
| 種交差性 | 交差種: Mouse, Rat, Human 交差が予測される動物種: Cow  |
| 免疫原 | Synthetic peptide corresponding to Human PACT (PKR activating protein)/ PRKRA aa 100-200 conjugated to keyhole limpet haemocyanin. (Peptide available as ab30768) |
| ポジティブ・コントロール | WB: HEK-293T, K562, HepG2, HAP1 and PC12 whole cell lysates; Mouse testis tissue lysate. ICC/IF: HeLa cells. IP: Mouse testis tissue. |
| 特記事項 | <p>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.</p> <p>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</p> |

製品の特性

| | |
|-------|---|
| 製品の状態 | Liquid |
| 保存方法 | 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. |
| バッファー | 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. |
| 精製度 | Immunogen affinity purified |
| ポリ/モノ | ポリクローナル |
| アイソタイプ | IgG |

アプリケーション

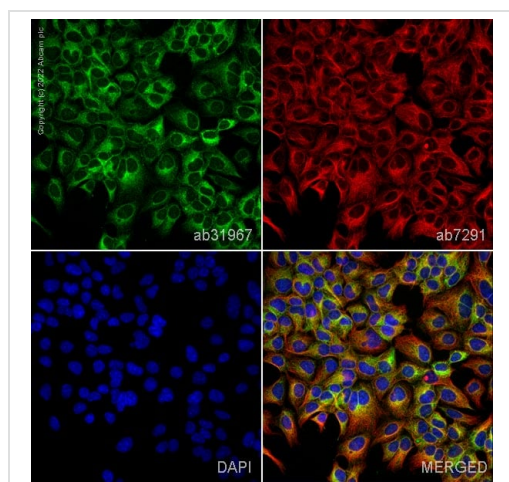
The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab31967の使用に適用されます**
 アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

| アプリケーション | Abreviews | 特記事項 |
|----------|-----------|--|
| 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. |

ターゲット情報

| | |
|-------|--|
| 機能 | Activates EIF2AK2/PKR in the absence of double stranded RNA (dsRNA), leading to phosphorylation of EIF2S1/EIF2-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. |
| 関連疾患 | 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. |
| 配列類似性 | Belongs to the PRKRA family. Contains 3 DRBM (double-stranded RNA-binding) domains. |
| ドメイン | 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. |
| 翻訳後修飾 | 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. |
| 細胞内局在 | Cytoplasm > perinuclear region. |

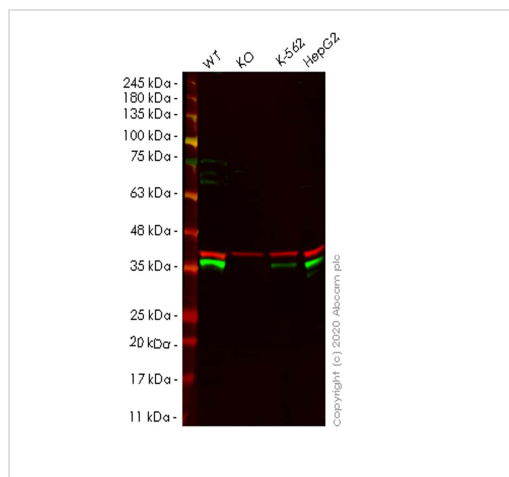
画像



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

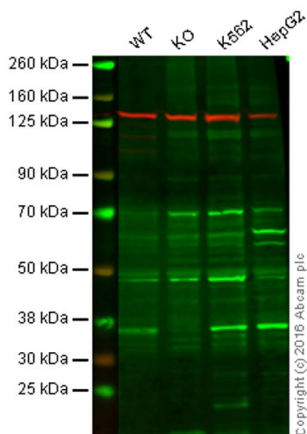
Predicted band size: 34 kDa

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)

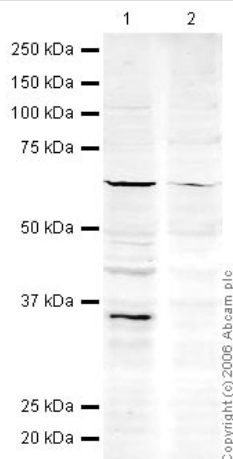
Lane 2: PACT (PKR activating protein)/PRKRA knockout HAP1 cell lysate (20 µg)

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

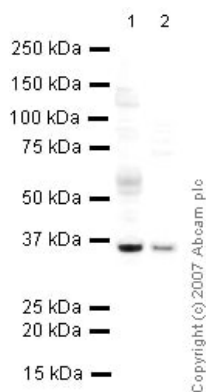
All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/15000 dilution

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

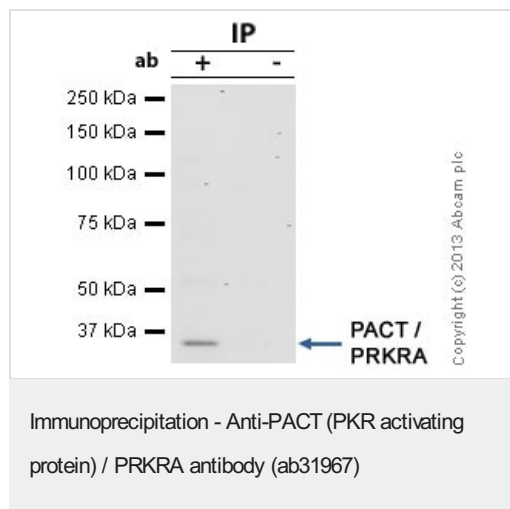
All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

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.



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°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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