# abcam

# Product datasheet

# Anti-PKC antibody [EPR17368] ab181558

יעלאעבע RabMAb

3 References 画像数 16

#### 製品の概要

製品名 Anti-PKC antibody [EPR17368]

製品の詳細 Rabbit monoclonal [EPR17368] to PKC

由来種 Rabbit

アプリケーション 適用あり: IHC-P, WB, ICC/IF, IP, Flow Cyt (Intra)

種交差性 交差種: Mouse, Rat, Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: Active human PKC beta 2, PKC alpha, PKC zeta and PKC iota full length proteins; A431,

> HeLa, 293T, C6, NIH/3T3, RAW 264.7 and PC-12 whole cell lysates; Human fetal kidney and fetal brain lysates; Mouse spleen, pancreas and lung lysates; Rat spleen and pancreas lysates. IHC-P: Human kidney, Human gastric adenocarcinoma, mouse testis and rat kidney tissues. ICC/IF:

A431 cells. Flow Cyt (intra): NIH/3T3 cells. IP: A431 whole cell extract.

特記事項

Recombinant PKC isoforms were tested for reactivity by western blot, and are marked as positive (+) or negative (-).

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  $\mathsf{RabMAb}^{\texttt{®}}$  technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

## 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 long

term. Avoid freeze / thaw cycle.

パッファー Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

**ポリた**ノ モノクローナル **クローン名** EPR17368

アイソタイプ lgG

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab181558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
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/2000. Detects a band of approximately 75 kDa (predicted molecular weight: 68 kDa).
ICC/IF		1/500.
IP		1/100.
Flow Cyt (Intra)		1/410.

#### ターゲット情報

## 機能

Calcium-activated, phospholipid- and diacylglycerol (DAG)-dependent serine/threonine-protein kinase that is involved in positive and negative regulation of cell proliferation, apoptosis, differentiation, migration and adhesion, tumorigenesis, cardiac hypertrophy, angiogenesis, platelet function and inflammation, by directly phosphorylating targets such as RAF1, BCL2, CSPG4, TNNT2/CTNT, or activating signaling cascade involving MAPK1/3 (ERK1/2) and RAP1GAP. Involved in cell proliferation and cell growth arrest by positive and negative regulation of the cell cycle. Can promote cell growth by phosphorylating and activating RAF1, which mediates the activation of the MAPK/ERK signaling cascade, and/or by up-regulating CDKN1A, which facilitates active cyclin-dependent kinase (CDK) complex formation in glioma cells. In intestinal cells stimulated by the phorbol ester PMA, can trigger a cell cycle arrest program which is associated with the accumulation of the hyper-phosphorylated growth-suppressive form of RB1 and induction of the CDK inhibitors CDKN1A and CDKN1B. Exhibits anti-apoptotic function in glioma cells and protects them from apoptosis by suppressing the p53/TP53-mediated activation of IGFBP3, and in leukemia cells mediates anti-apoptotic action by phosphorylating BCL2. During macrophage differentiation induced by macrophage colony-stimulating factor (CSF1), is translocated to the nucleus and is associated with macrophage development. After wounding, translocates from focal contacts to lamellipodia and participates in the modulation of desmosomal adhesion. Plays a role in cell motility by phosphorylating CSPG4, which induces association of CSPG4 with extensive lamellipodia at the cell periphery and polarization of the cell accompanied by increases in cell motility. Is highly expressed in a number of cancer cells where it can act as a

tumor promoter and is implicated in malignant phenotypes of several tumors such as gliomas and breast cancers. Negatively regulates myocardial contractility and positively regulates angiogenesis, platelet aggregation and thrombus formation in arteries. Mediates hypertrophic growth of neonatal cardiomyocytes, in part through a MAPK1/3 (ERK1/2)-dependent signaling pathway, and upon PMA treatment, is required to induce cardiomyocyte hypertrophy up to heart failure and death, by increasing protein synthesis, protein-DNA ratio and cell surface area. Regulates cardiomyocyte function by phosphorylating cardiac troponin T (TNNT2/CTNT), which induces significant reduction in actomyosin ATPase activity, myofilament calcium sensitivity and myocardial contractility. In angiogenesis, is required for full endothelial cell migration, adhesion to vitronectin (VTN), and vascular endothelial growth factor A (VEGFA)-dependent regulation of kinase activation and vascular tube formation. Involved in the stabilization of VEGFA mRNA at post-transcriptional level and mediates VEGFA-induced cell proliferation. In the regulation of calcium-induced platelet aggregation, mediates signals from the CD36/GP4 receptor for granule release, and activates the integrin heterodimer ITGA2B-ITGB3 through the RAP1GAP pathway for adhesion. During response to lipopolysaccharides (LPS), may regulate selective LPS-induced macrophage functions involved in host defense and inflammation. But in some inflammatory responses, may negatively regulate NF-kappa-B-induced genes, through IL1A-dependent induction of NF-kappa-B inhibitor alpha (NFKBIA/IKBA). Upon stimulation with 12-Otetradecanoylphorbol-13-acetate (TPA), phosphorylates EIF4G1, which modulates EIF4G1 binding to MKNK1 and may be involved in the regulation of EIF4E phosphorylation. Phosphorylates KIT, leading to inhibition of KIT activity. Phosphorylates ATF2 which promotes cooperation between ATF2 and JUN, activating transcription.

### 配列類似性

Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. PKC subfamily. Contains 1 AGC-kinase C-terminal domain.

Contains 1 C2 domain.

Contains 2 phorbol-ester/DAG-type zinc fingers.

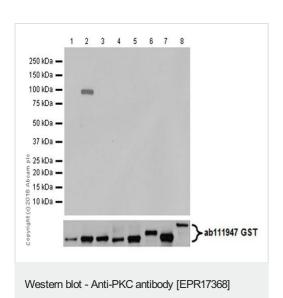
Contains 1 protein kinase domain.

## 細胞内局在

(ab181558)

Cytoplasm. Cell membrane. Mitochondrion membrane. Nucleus.

# 画像



**All lanes :** Anti-PKC antibody [EPR17368] (ab181558) at 1/2000 dilution

Lane 1: Active human PKC beta 1 full length protein

Lane 2: Active human PKC beta 2 full length protein

Lane 3: Active human PKC gamma full length protein

Lane 4: Active human PKC delta full length protein

Lane 5: Active human PKC eta full length protein

Lane 6: Active human PKC epsilon full length protein

Lane 7: Active human PKC theta full length protein

Lane 8 : Active human PKC mu full length protein

Lysates/proteins at 0.02 µg per lane.

#### Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000

Predicted band size: 68 kDa

Exposure time: 2 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time:2 seconds

Active human PKC beta 1 full length protein (Catalog#ab60840) contains aa1-671 with GST-tag; Active human PKC beta 2 full length protein (Catalog#ab60841) contains aa1-673 with GST-tag; Active human PKC gamma full length protein (Catalog#ab60842) contains aa1-677 with GST-tag; Active human PKC delta full length protein (Catalog#ab60844) contains aa1-676 with GST-tag; Active human PKC eta full length protein (Catalog#ab60849) contains aa1-683 with GST-tag; Active human PKC epsilon full length protein (Catalog#ab60847) contains aa1-737 with GST-tag; Active human PKC theta full length protein (Catalog#ab56641) contains aa1-706 with GST-tag; Active human PKC mu full length protein (Catalog#ab60873) contains aa1-912 with GST-tag.

1 2 3

250 KDa —
150 KDa —
100 KDa —
75 KDa —
50 KDa —
37 KDa —
37 KDa —
91 15 KDa —
91 15

Western blot - Anti-PKC antibody [EPR17368] (ab181558)

**All lanes :** Anti-PKC antibody [EPR17368] (ab181558) at 1/2000 dilution

Lane 1: Active human PKC alpha full length protein

Lane 2: Active human PKC zeta full length protein

Lane 3: Active human PKC iota full length protein

Lysates/proteins at 0.02 µg per lane.

## Secondary

All lanes: Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/50000

dilution

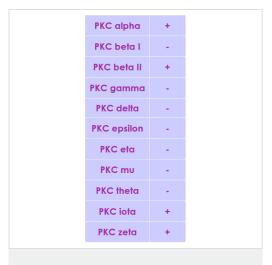
Predicted band size: 68 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1: 10 seconds; Lane 2 and 3: 3 seconds.

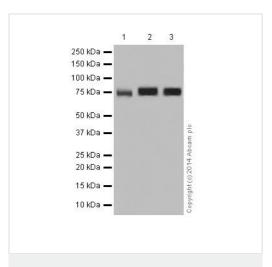
Active human PKC alpha full length protein (Catalog#ab55672)

contains aa1-672 with GST-tag; Active human PKC zeta full length protein (Catalog# <u>ab60848</u>) contains aa1-592 with GST-tag; Active human PKC iota full length protein (Catalog#<u>ab60850</u>) contains aa1-596 with GST-tag.



Recombinant PKC isoforms were tested for reactivity by western blot, and are marked as positive (+) or negative (-).

Western blot - Anti-PKC antibody [EPR17368] (ab181558)



Western blot - Anti-PKC antibody [EPR17368] (ab181558)

**All lanes :** Anti-PKC antibody [EPR17368] (ab181558) at 1/2000 dilution

**Lane 1 :** A431 (Human epidermoid carcinoma cell line) whole cell lysate

**Lane 2 :** HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 3**: 293T (Human epithelial cell line from embryonic kidney) whole cell lysate

Lysates/proteins at 20 µg per lane.

## Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 68 kDa **Observed band size:** 75 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PKC antibody [EPR17368] (ab181558)

**All lanes :** Anti-PKC antibody [EPR17368] (ab181558) at 1/10000 dilution

Lane 1 : Human fetal kidney lysate

Lane 2 : Human fetal brain lysate

Lysates/proteins at 10 µg per lane.

# Secondary

**All lanes :** Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/1000 dilution

**Predicted band size:** 68 kDa **Observed band size:** 75 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PKC antibody [EPR17368] (ab181558)

**All lanes :** Anti-PKC antibody [EPR17368] (ab181558) at 1/10000 dilution

Lane 1 : Mouse spleen lysate

Lane 2 : Rat spleen lysate

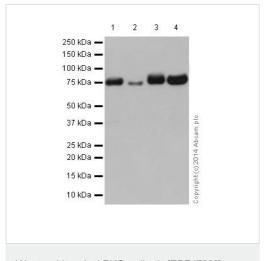
Lysates/proteins at 10 µg per lane.

## Secondary

**All lanes :** Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 68 kDa Observed band size: 75 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PKC antibody [EPR17368] (ab181558)

All lanes: Anti-PKC antibody [EPR17368] (ab181558) at 1/2000 dilution

Lane 1: C6 (Rat glial tumor cell line) whole cell lysate

Lane 2: RAW 264.7 (Mouse macrophage cell line transformed

with Abelson murine leukemia virus) whole cell lysate

Lane 3: PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 4: NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

# **Secondary**

All lanes: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 68 kDa

Observed band size: 75 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

250 kDa 🕳 150 kDa -100 kDa -75 kDa -50 kDa -37 kDa -25 kDa 🕳 20 kDa 🕳 15 kDa 🕳 10 kDa 🕳

Western blot - Anti-PKC antibody [EPR17368] (ab181558)

All lanes: Anti-PKC antibody [EPR17368] (ab181558) at 1/50000 dilution

Lane 1: Rat pancreas lysate

Lane 2: Mouse pancreas lysate

Lane 3: Mouse lung lysate

Lysates/proteins at 10 µg per lane.

# **Secondary**

All lanes: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 68 kDa Observed band size: 75 kDa Blocking/Dilution buffer: 5% NFDM/TBST.

Negative control

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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC antibody
[EPR17368] (ab181558)

Immunohistochemical analysis of paraffinembedded Human kidney tissue labeling PKC (various isoforms) with ab181558 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic and weak nuclear staining on glomerular and renal tubule is observed.

#### Reference:

1: Y Mizukam,T Hirata,et.al.(1997) Nuclear translocation of PKC zeta during ischemia and its inhibition by wortmannin, an inhibitor of phosphatidylinositol

2: Jose A. Crespo,Petra Stockl,et.al(2012) Activation of PKCzeta and PKMzeta in the Nucleus Accumbens Core Is Necessary for the Retrieval,Consolidation and Reconsolidation of Drug Memory.Plos One 7(2):e30502.

3-kinase.FEBS Lett 401 (2-3): 247-251.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab97051</u> at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

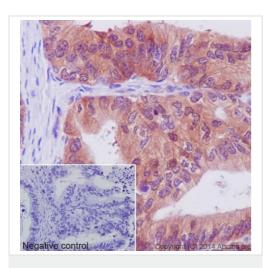
Immunohistochemical analysis of paraffin-embedded Human gastric adenocarcinoma tissue labeling PKC (various isoforms) with ab181558 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic and weak nuclear staining on Human gastric adenocarcinoma is observed.

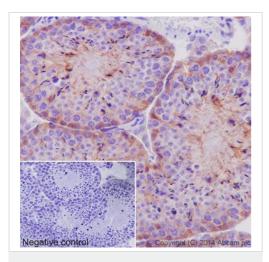
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC antibody
[EPR17368] (ab181558)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC antibody
[EPR17368] (ab181558)

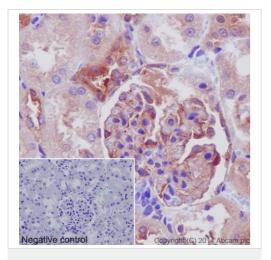
Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling PKC (various isoforms) with ab181558 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic staining on testis seminiferous tubule is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC antibody
[EPR17368] (ab181558)

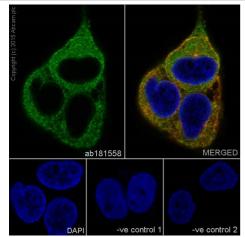
Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labeling PKC (various isoforms) with ab181558 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic and weak nuclear staining on glomerular and renal tubule is observed.

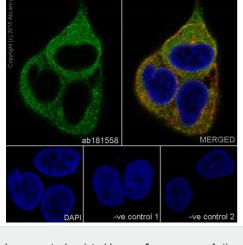
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-PKC antibody [EPR17368] (ab181558)



The negative controls are as follows:

dilution (red).

nuclear counter stain is DAPI (blue).

-ve control 1: ab181558 at 1/500 dilution followed by ab150120 at 1/500 dilution.

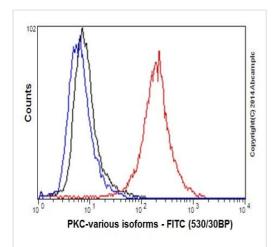
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A431 (Human epidermoid carcinoma cell line) cells labeling PKC (various isoforms) with ab181558 at 1/500 dilution, followed by Goat Anti-Rabbit lgG (Alexa Fluor<sup>®</sup> 488)

Confocal image showing cytoplasm staining on A431 cell line. The

(ab150077) secondary antibody at 1/400 dilution (green).

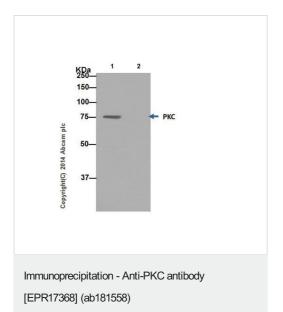
Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] -Loading Control (ab7291) at 1/1000 dilution and Goat Anti-Mouse lgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) at 1/1000

-ve control 2: ab7291 at 1/500 dilution followed by ab150077 at 1/400 dilution.



Flow Cytometry (Intracellular) - Anti-PKC antibody [EPR17368] (ab181558)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling PKC (various isoforms)with ab181558 at 1/410 dilution (red) compared with a Rabbit lgG,monoclonal[EPR25A]-lsotype control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit lgG (FITC)at 1/150 dilution was used as the secondary antibody.

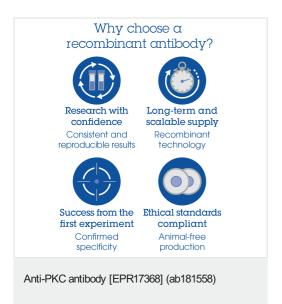


PKC was immunoprecipitated from 1 mg of A431 (Human epidermoid carcinoma cell line) whole cell extract with ab181558 at 1/100 dilution. Western blot was performed from the immunoprecipitate using ab181558 at 1/5000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG was used as secondary antibody at 1/1500 dilution.

Lane 1: A431 whole cell extract.

Lane 2: PBS.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.



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