abcam

Product datasheet

Anti-PKC delta antibody [EPR17075] ab182126



★★★★★ 7 Abreviews 50 References 画像数 16

製品の概要

製品名 Anti-PKC delta antibody [EPR17075]

製品の詳細 Rabbit monoclonal [EPR17075] to PKC delta

由来種 Rabbit

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

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: HEK-239T, A431, C6, NIH/3T3, HAP1 and HeLa whole cell lysates, human fetal brain and

> fetal heart, mouse and rat thymus and brain and rat spleen tissue lysates. IHC-P: Human spleen, human transitional cell carcinoma of bladder, mouse liver and rat testis tissues. ICC/IF: Wild-type

HAP1 (PMA-treated and untreated) and HeLa cells. Flow Cyt (intra): HeLa cells.

特記事項 Protein kinase C delta type (PKCD) is an enzyme encoded for by the PRKCD gene and is

> expressed ubiquitously among cells and tissues. It is thought that PKCD, when activated in distinct ways, plays critical roles in cellular functions such as the control of growth, differentiation

and apoptosis.

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**.

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.

バッファー Preservative: 0.01% Sodium azide

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

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 EPR17075

アイソタイプ IgG

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ab182126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
Flow Cyt (Intra)		1/250. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★★ (4)	1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★ 市 市 市 市 (1)	1/5000. Detects a band of approximately 40, 78 kDa (predicted molecular weight: 78 kDa).
ICC/IF		Use a concentration of 5 μ g/ml. Treatment with 10nM PMA for 10 min induces translocation of PKC δ to the membrane.

ターゲット情報

機能 This is calcium-independent, phospholipid-dependent, serine- and threonine-specific enzyme.

PKC is activated by diacylglycerol which in turn phosphorylates a range of cellular proteins. PKC also serves as the receptor for phorbol esters, a class of tumor promoters. May play a role in antigen-dependent control of B-cell function. Phosphorylates MUC1 in the C-terminal and

regulates the interaction between MUC1 and beta-catenin.

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.

ドメイン The C1 domain, containing the phorbol ester/DAG-type region 1 (C1A) and 2 (C1B), is the

diacylglycerol sensor.

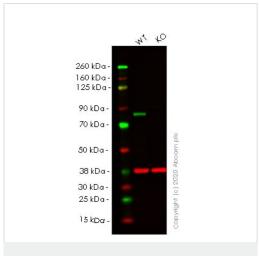
The C2 domain is a non-calcium binding domain. It binds proteins containing phosphotyrosine in

a sequence-specific manner.

翻訳後修飾 Phosphorylated on Thr-507, within the activation loop. Autophosphorylated and/or phosphorylated.

Although the Thr-507 phosphorylation occurs it is not a prerequisite for enzymatic activity.

細胞内局在 Cytoplasm. Membrane.



Western blot - Anti-PKC delta antibody [EPR17075] (ab182126)

Lane 1: Wild-type HeLa cell lysate

Lane 2: PRKCD knockout HeLa cell lysate

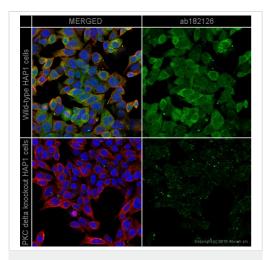
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 78 kDa **Observed band size:** 80 kDa

Lanes 1-2: Merged signal (red and green). Green - ab182126 observed at 80 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab182126 was shown to react with PKC delta in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265721 (knockout cell lysate ab257043) was used. Wild-type HeLa and PKC delta knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab182126 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 5000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

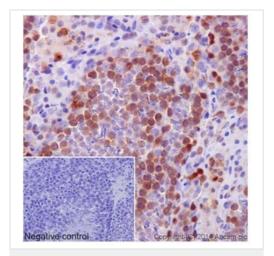


Immunocytochemistry/ Immunofluorescence - Anti-PKC delta antibody [EPR17075] (ab182126)

ab182126 staining PKC δ in untreated wild-type HAP1 cells (top panel) and PKC δ untreated knockout HAP1 cells (bottom panel). Untreated cells show PKC δ being expressed in the cytoplasm. The cells were fixed with 4% formaldehyde (10min), 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 ab182126 at 1/200 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 lgG (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 100% methanol (5 min).

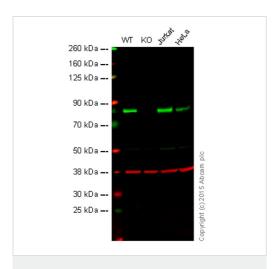
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC delta antibody
[EPR17075] (ab182126)

Immunohistochemical analysis of paraffin-embedded rat testis tissue labeling PKC delta with ab182126 at 1/2000 dilution, followed by **Anti-Rabbit HRP** (**ab97051**) at 1/500 dilution. Cytoplasmic and weak nuclear staining on cells in the seminiferous tubule of rat testis is detected. The negative control utilised PBS instead of primary antibody. Counter stained with Hematoxylin.

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



Western blot - Anti-PKC delta antibody [EPR17075] (ab182126)

Lane 1: Wild-type HAP1 cell lysate

Lane 2: PKC delta knockout HAP1 cell lysate

Lane 3 : Jurkat cell lysate

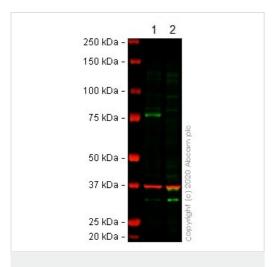
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 78 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab182126 observed at 78 kDa. Red - loading control, **ab8226**, observed at 42 kDa.

ab182126 was shown to specifically react with PKC delta when PKC delta knockout samples were used. Wild-type and PKC delta knockout samples were subjected to SDS-PAGE. ab182126 and ab8226 (loading control to beta Actin) were diluted to 1/5000 and 1/1000 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.



Western blot - Anti-PKC delta antibody [EPR17075] (ab182126)

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: PRKCD CRISPR/Cas9 edited HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

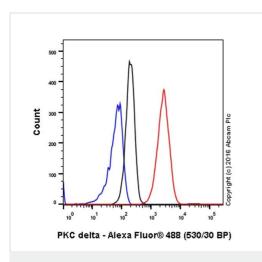
Performed under reducing conditions.

Predicted band size: 78 kDa **Observed band size:** 78 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab182126 observed at 78 kDa. Red - loading control, <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

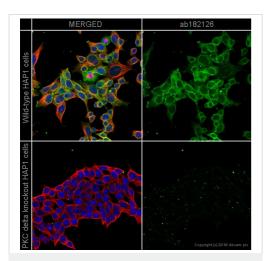
ab182126 was shown to react with PKC in wild-type HEK-293T cells in western blot. The bands observed in PRKCD CRISPR/Cas9 edited cell line ab266143 (PRKCD CRISPR/Cas9 edited cell lysate ab257042) below 78kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and PRKCD CRISPR/Cas9 edited HEK-293T cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab182126 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4

176;® 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-PKC delta antibody [EPR17075] (ab182126)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling PKC delta with purified ab182126 at 1/250 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, cellswithout incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.

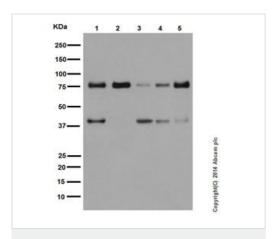


Immunocytochemistry/ Immunofluorescence - Anti-PKC delta antibody [EPR17075] (ab182126)

ab182126 staining PKC δ in 10nM PMA-treated wild-type HAP1 cells (top panel) and PKC δ in 10nM PMA-treated knockout HAP1 cells (bottom panel). The cells were treated with 10nM PMA for 10 minutes to induce translocation of PKC δ to the cell membrane. The cells were then fixed with 4% formaldehyde (10min), 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 ab182126 at 1/200 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 μ g/ml (shown in green) and a goat secondary antibody to Mouse lgG (Alexa Fluor® 594) (ab150117) at 2 μ g/ml (shown in pseudocolor 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 100% methanol (5 min).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Western blot - Anti-PKC delta antibody [EPR17075] (ab182126)

Lane 1: Mouse brain lysates

Lane 2: Rat brain lysates

Lane 3: Rat spleen lysates

Lane 4: C6 (Rat glial tumor cells) whole cell lysates

Lane 5: NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysates

Lysates/proteins at 10 µg per lane.

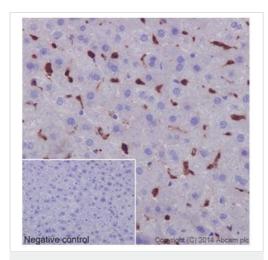
Secondary

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

Predicted band size: 78 kDa

The 40kDa band represents the cleaved kinase domain.

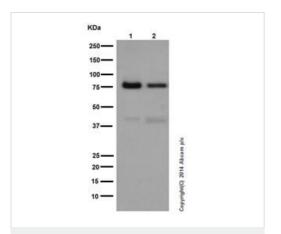
Blocking/dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC delta antibody
[EPR17075] (ab182126)

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling PKC delta with ab182126 at 1/2000 dilution, followed by **Anti-Rabbit HRP** (**ab97051**) at 1/500 dilution. Cytoplasmic and nuclear staining on kupffer cells of Mouse liver is detected. The negative control utilised PBS instead of primary antibody. Counter stained with Hematoxylin.

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



Western blot - Anti-PKC delta antibody [EPR17075] (ab182126)

All lanes : Anti-PKC delta antibody [EPR17075] (ab182126) at 1/5000 dilution

Lane 1 : Human fetal brain lysates
Lane 2 : Human fetal heart lysates

Lysates/proteins at 10 µg per lane.

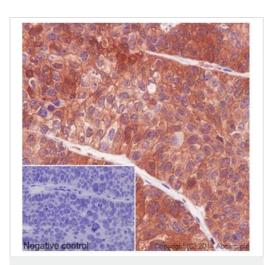
Secondary

All lanes : Anti-Rabbit $\lg G$ (HRP), specific to the non-reduced form of $\lg G$ at 1/1000 dilution

Predicted band size: 78 kDa

The 40kDa band represents the cleaved kinase domain.

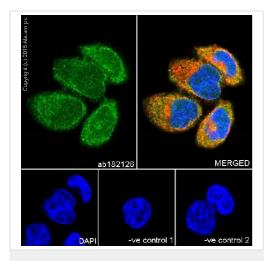
Blocking/dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC delta antibody
[EPR17075] (ab182126)

Immunohistochemical analysis of paraffin-embedded Human transitional cell carcinoma of bladder tissue labeling PKC delta with ab182126 at 1/2000 dilution, followed by **Anti-Rabbit HRP** (ab97051) at 1/500 dilution. Cytoplasmic and weak nuclear staining on cancer cells of bladder transitional cell carcinoma is detected. The negative control utilised PBS instead of primary antibody. Counter stained with Hematoxylin.

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

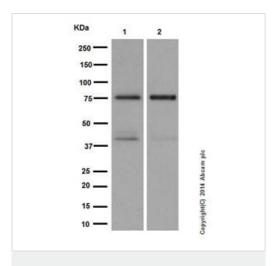


Immunocytochemistry/ Immunofluorescence - Anti-PKC delta antibody [EPR17075] (ab182126)

Immunofluorescent analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling PKC delta with ab182126 at 1/100 dilution. The cells were permeabilised with 0.1% Triton X-100. Goat anti-rabbit IAlexa Fluor[®] 488 (lgG) (ab150077) at 1/400 dilution was used as the secondary antibody (green). The confocal image shows both cytoplasmic and nuclear staining on HeLa cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/500 and ab150120 (goat anti-mouse AlexaFluor[®] 594 secondary antibody) at 1/500 dilution (red).

The negative controls are as follows;

- ab182126 at 1/100 dilution followed by <u>ab150120</u> (Alexa Fluor[®]
 Goat anti-Mouse secondary) at 1/500 dilution.
- 2. <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/500 dilution followed by <u>ab150077</u> (Alexa Fluor[®] 488 Goat Anti-Rabbit lgG H&L) at 1/400 dilution.



Western blot - Anti-PKC delta antibody [EPR17075] (ab182126)

Lane 1: A431 (Human epidermoid carcinoma) whole cell lysates at 10 μg

Lane 2: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates at 20 µg

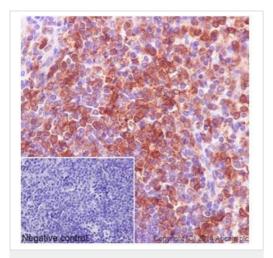
Secondary

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

Predicted band size: 78 kDa

The 40kDa band represents the cleaved kinase domain.

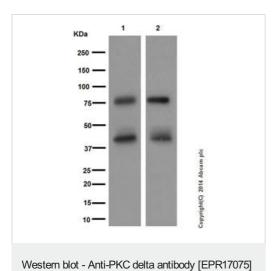
Blocking/dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC delta antibody
[EPR17075] (ab182126)

Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling PKC delta with ab182126 at 1/2000 dilution, followed by **Anti-Rabbit HRP** (**ab97051**) at 1/500 dilution. Cytoplasmic and nucleus staining on lymphocytes of Human spleen is detected. The negative control utilised PBS instead of primary antibody. Counter stained with Hematoxylin.

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



(ab182126)

All lanes : Anti-PKC delta antibody [EPR17075] (ab182126) at 1/100000 dilution

Lane 1: Mouse thymus lysates

Lane 2: Rat thymus lysates

Lysates/proteins at 10 mg/ml per lane.

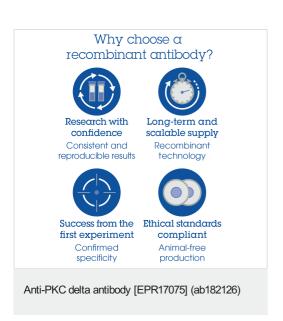
Secondary

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

Predicted band size: 78 kDa

The 40kDa band represents the cleaved kinase domain.

Blocking/dilution buffer: 5% NFDM/TBST.



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