abcam

Product datasheet

Anti-PKC delta antibody [EPR17075] - BSA and Azide free ab222229

KO VALIDATED Recombinant RabMAb

12 Images

Overview

Product name	Anti-PKC delta antibody [EPR17075] - BSA and Azide free
Description	Rabbit monoclonal [EPR17075] to PKC delta - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293T, Hap1, A431, C6, NIH/3T3, Jurkat 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.
General notes	ab222229 is the carrier-free version of **ab182126**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17075
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab222229 in the following tested applications.

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

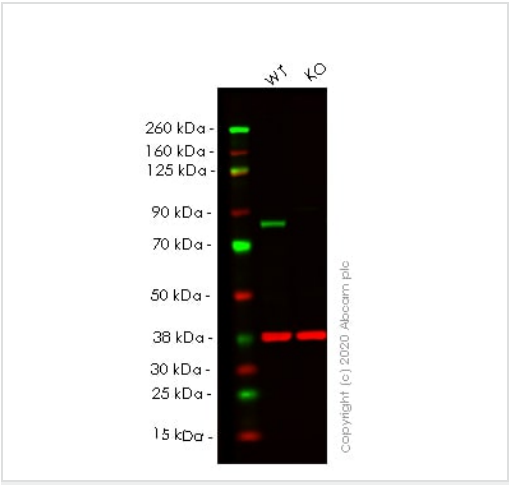
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 40, 78 kDa (predicted molecular weight: 78 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration. Treatment with 10nM PMA for 10 min induces translocation of PKC? to the membrane.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

Function	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.
Sequence similarities	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.
Domain	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.
Post-translational modifications	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.
Cellular localization	Cytoplasm. Membrane.

Images



Western blot - Anti-PKC delta antibody [EPR17075] - BSA and Azide free (ab222229)

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

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.

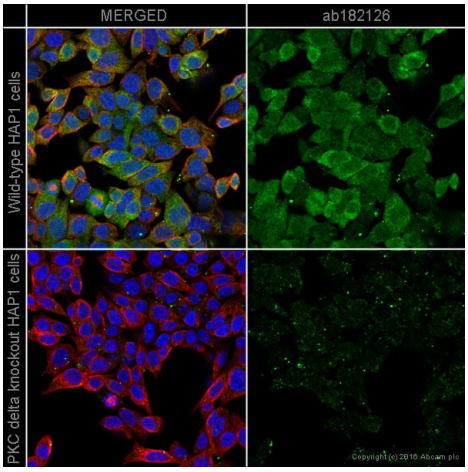
Predicted band size: 78 kDa
Observed band size: 80 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab182126**).

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 PRKCD 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 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.



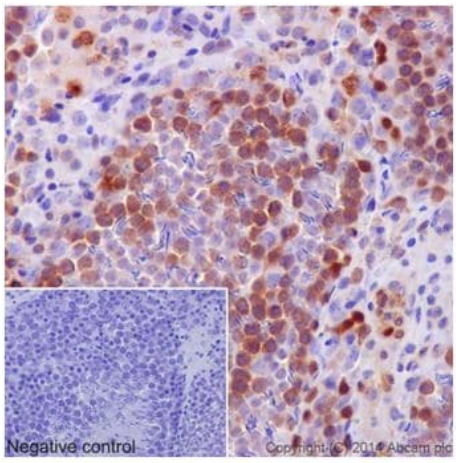
Immunocytochemistry/ Immunofluorescence - Anti-PKC delta antibody [EPR17075] - BSA and Azide free (ab222229)

ab182126 staining PKCδ in untreated wild-type HAP1 cells (top panel) and PKCδ untreated knockout HAP1 cells (bottom panel). Untreated cells show PKCδ 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 IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to Mouse IgG (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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182126**).

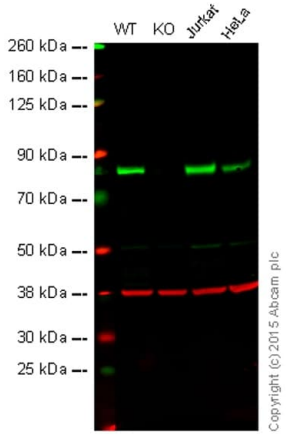


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC delta antibody [EPR17075] - BSA and Azide free (ab222229)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182126**).

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] - BSA and Azide free (ab222229)

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

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

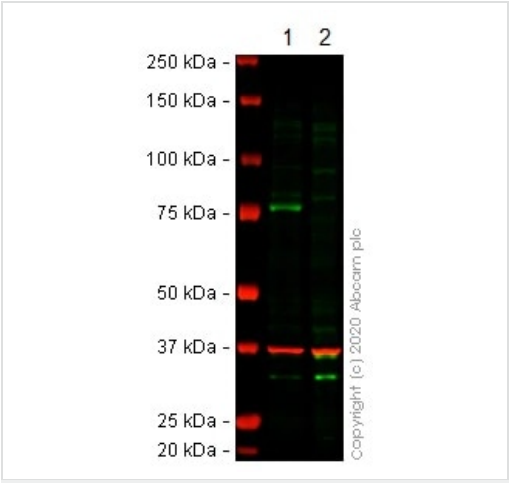
Lysates/proteins at 20 µg per lane.

Predicted band size: 78 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab182126**).

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] - BSA and Azide free (ab222229)

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

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.

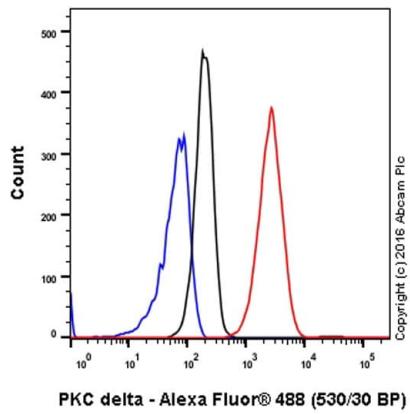
Predicted band size: 78 kDa
Observed band size: 78 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab182126**).

Lanes 1 - 2: Merged signal (red and green). Green - **ab182126** observed at 78 kDa. Red - loading control, **ab8245** (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

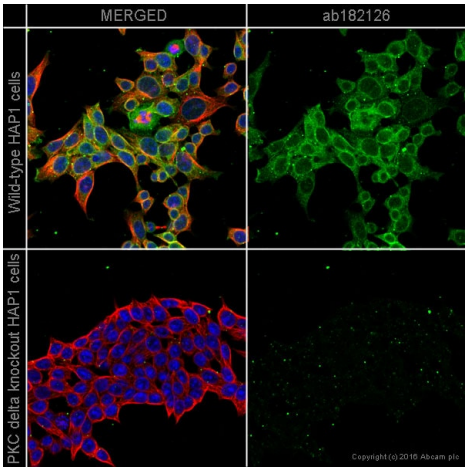
176;® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-PKC delta antibody [EPR17075] - BSA and Azide free (ab222229)

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 IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182126**).



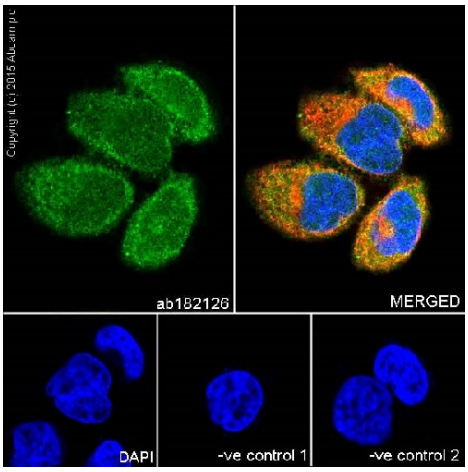
Immunocytochemistry/ Immunofluorescence - Anti-PKC delta antibody [EPR17075] - BSA and Azide free (ab222229)

ab182126 staining PKCδ in 10nM PMA-treated wild-type HAP1 cells (top panel) and PKCδ in 10nM PMA-treated knockout HAP1 cells (bottom panel). The cells were treated with 10nM PMA for 10 minutes to induce translocation of PKCδ 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 IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to Mouse IgG (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).

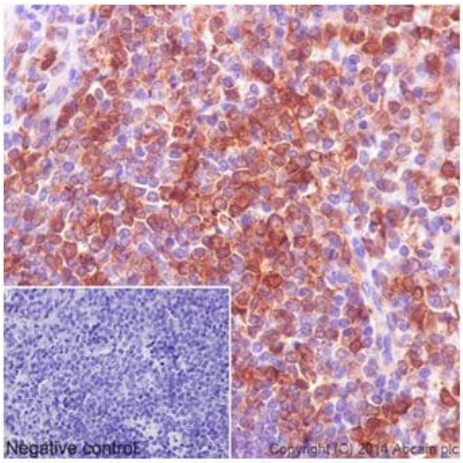
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182126**).



Immunocytochemistry/ Immunofluorescence - Anti-PKC delta antibody [EPR17075] - BSA and Azide free (ab222229)

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 (IgG) (**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;
1. **ab182126** at 1/100 dilution followed by **ab150120** (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/500 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution followed by **ab150077** (Alexa Fluor® 488 Goat Anti-Rabbit IgG H&L) at 1/400 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182126**).

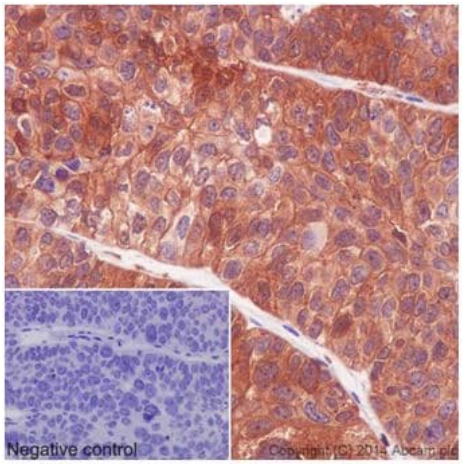


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC delta antibody [EPR17075] - BSA and Azide free (ab222229)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182126**).

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

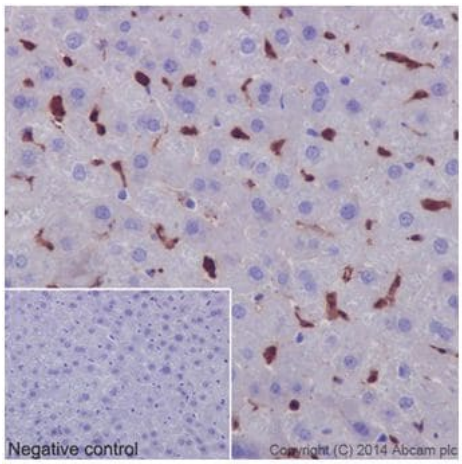


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC delta antibody [EPR17075] - BSA and Azide free (ab222229)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182126**).

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

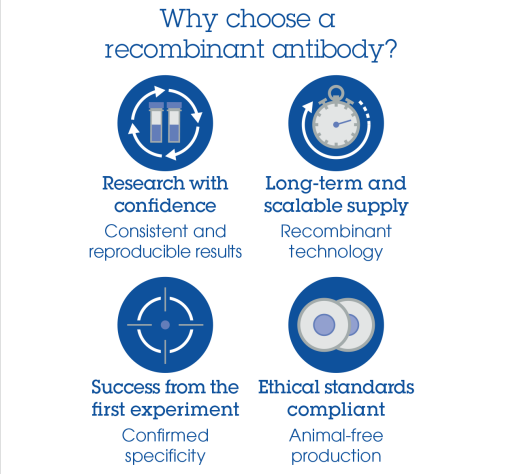


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC delta antibody [EPR17075] - BSA and Azide free (ab222229)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182126**).

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



Anti-PKC delta antibody [EPR17075] - BSA and Azide free (ab222229)

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