**Product datasheet**

**Anti-PKC delta antibody [EPR17075] ab182126**

[Image]

**Overview**

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-PKC delta antibody [EPR17075]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Rabbit monoclonal [EPR17075] to PKC delta</td>
</tr>
<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Tested applications</td>
<td><strong>Suitable for:</strong> Flow Cyt, IHC-P, WB, ICC/IF</td>
</tr>
<tr>
<td>Species reactivity</td>
<td>Reacts with: Mouse, Rat, Human</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Recombinant fragment within Mouse PKC delta aa 500 to the C-terminus. The exact sequence is proprietary. Database link: P28867</td>
</tr>
</tbody>
</table>

**Positive control**

- WB: A431, C6, NIH/3T3 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: HeLa cells.

**General notes**

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents. This product is a recombinant rabbit monoclonal antibody.

**Properties**

<table>
<thead>
<tr>
<th>Form</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage buffer</td>
<td>Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA</td>
</tr>
<tr>
<td>Purity</td>
<td>Protein A purified</td>
</tr>
<tr>
<td>Clonality</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Clone number</td>
<td>EPR17075</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
</tbody>
</table>

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**Product datasheet**

**Anti-PKC delta antibody [EPR17075] ab182126**

[Image]
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.

Applications

Our Abpromise guarantee covers the use of ab182126 in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/250. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>WB</td>
<td></td>
<td>1/5000. Detects a band of approximately 40, 78 kDa (predicted molecular weight: 78 kDa).</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use a concentration of 5 µg/ml. Treatment with 10nM PMA for 10 min induces translocation of PKCδ to the membrane.</td>
</tr>
</tbody>
</table>

Target

Target Images 2
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: PKC delta knockout HAP1 cell lysate (20 µg)
Lane 3: Jurkat cell lysate (20 µg)
Lane 4: HeLa cell lysate (20 µg)

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

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).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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.
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.
Immunocytochemistry/ Immunofluorescence - Anti-PKC delta antibody [EPR17075] (ab182126)

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 2μg/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).
Western blot - Anti-PKC delta antibody [EPR17075] (ab182126)

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

- **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 embryo 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.
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.

**Western blot**

**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 IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

**Predicted band size** : 78 kDa

The 40kDa band represents the cleaved kinase domain.

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

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

**All lanes:** Anti-PKC delta antibody [EPR17075] (ab182126) at 1/10000 dilution

**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 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.
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.

**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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