

Product datasheet

Anti-PKC delta (phospho S645) antibody [EP1486Y] ab108972

KO VALIDATED Recombinant RabMAB[®]

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Overview

Product name	Anti-PKC delta (phospho S645) antibody [EP1486Y]
Description	Rabbit monoclonal [EP1486Y] to PKC delta (phospho S645)
Host species	Rabbit
Tested applications	Suitable for: ELISA, Dot blot, WB, IHC-P Unsuitable for: Flow Cyt, ICC/IF or IP
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	293T cell lysate; Human spleen and bladder carcinoma; HeLa cells.
General notes	<p>This product has switched from a hybridoma to recombinant production method on 20th November 2023.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number EP1486Y
Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab108972 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
ELISA		Use at an assay dependent concentration.
Dot blot		1/1000.
WB		1/10000 - 1/50000. Predicted molecular weight: 78 kDa.
IHC-P		1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Application notes Is unsuitable for Flow Cyt, ICC/IF or IP.

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.

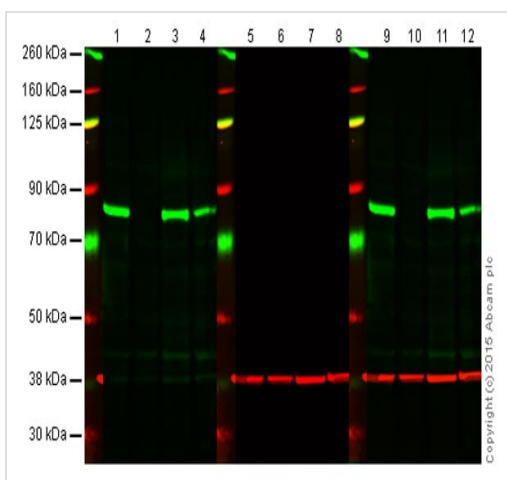
Sequence similarities Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. PKC subfamily. Contains 1 AGC-kinase C-terminal domain. Contains 1 C2 domain. Contains 2 phorbol-ester/DAG-type zinc fingers. Contains 1 protein kinase domain.

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.

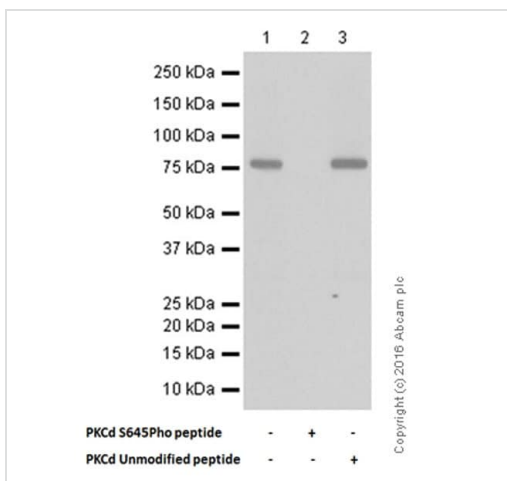
Images



Western blot - Anti-PKC delta (phospho S645) antibody [EP1486Y] (ab108972)

Lanes 1, 5 and 9: Wild-type HAP1 cell lysate (20 µg)
Lanes 2, 6 and 10: PKC delta knockout HAP1 cell lysate (20 µg)
Lanes 3, 7 and 11: A431 cell lysate (20 µg)
Lanes 4, 8 and 12: HeLa cell lysate (20 µg)
Lanes 1, 2, 3 and 4: Green signal from target – ab108972 observed at 78 kDa
Lanes 5, 6, 7 and 8: Red signal from loading control – **ab8245** observed at 37 kDa
Lanes 9, 10, 11 and 12: Merged (red and green) signal

ab108972 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. ab108972 and **ab8245** (loading control to GAPDH) were diluted 1/10000 and 1/2000 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-PKC delta (phospho S645) antibody [EP1486Y] (ab108972)

Primary ab Dilution 1:100,000 dilution, Secondary ab Goat Anti-Rabbit IgG, (H+L), HRP conjugated (**ab97051**), Secondary ab dilution 1:20,000 dilution,

Blocking buffer and concentration 5% NFDm/TBST, Diluting buffer and concentration 5% NFDm/TBST,

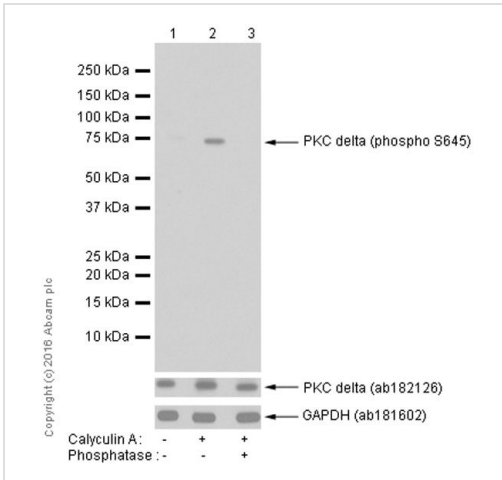
Lane 1: THP-1 whole cell lysate treated with Calyculin A with no peptides,

Lane 2: THP-1 whole cell lysate treated with Calyculin A with PKCd S645Pho peptides,

Lane 3: THP-1 whole cell lysate treated with Calyculin A with PKCd unmodified peptides,

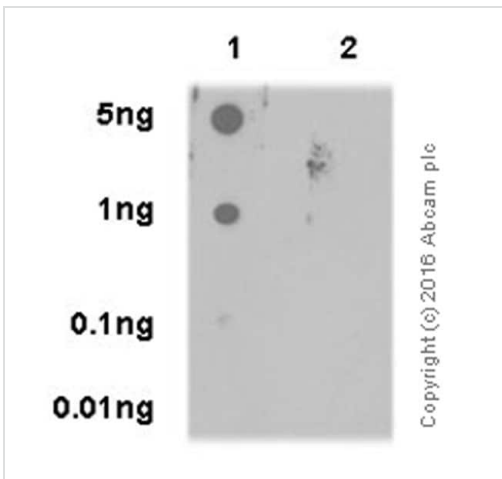
Observed MW 78 kDa,

Exposure time 10 seconds



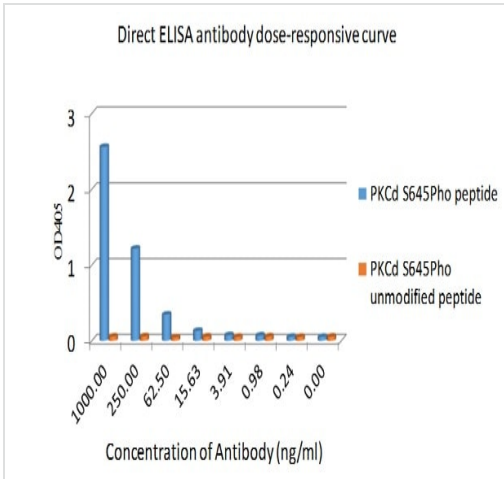
Western blot - Anti-PKC delta (phospho S645) antibody [EP1486Y] (ab108972)

Primary ab Dilution 1:100,000 dilution, Secondary ab description and code (ab id) Goat Anti-Rabbit IgG, (H+L), HRP conjugated (**ab97051**), Secondary ab dilution 1: 20,000 dilution, Blocking buffer and concentration 5% NFDm/TBST, Diluting buffer and concentration 5% NFDm/TBST, Lane 1: Untreated THP-1 (Human monocytic leukemia cell line) whole cell lysates 15ug, Lane 2: THP-1 (Human monocytic leukemia cell line) treated with 50nM Calyculin A for 60 minutes whole cell lysates 15ug. Lane 3: THP-1 (Human monocytic leukemia cell line) treated with 50nM Calyculin A for 60 minutes' whole cell lysates 15ug. Then the membrane was incubated with phosphatase. Lane 4: None, Observed MW 78 kDa, Exposure time 5 seconds



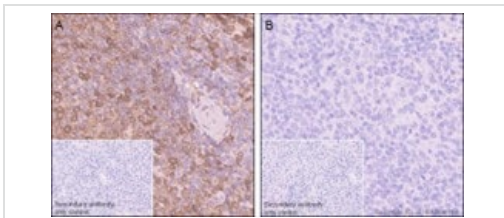
Dot Blot - Anti-PKC delta (phospho S645) antibody [EP1486Y] (ab108972)

Primary ab dilution 1: 1000 dilution (2.355 µg /ml), Secondary ab description and code (ab id) Goat Anti-Rabbit IgG, (H+L), HRP conjugated (**ab97051**), Secondary ab dilution 1:100,000 dilution, Blocking buffer and concentration 5% NFDm/TBST, Diluting buffer and concentration 5% NFDm /TBST, Lane 1: PKCd S645Pho peptides, Lane 2: PKCd unmodified peptides, Lane 3: None, Lane 4: None, Observed MW N/A, Exposure time 3 minutes



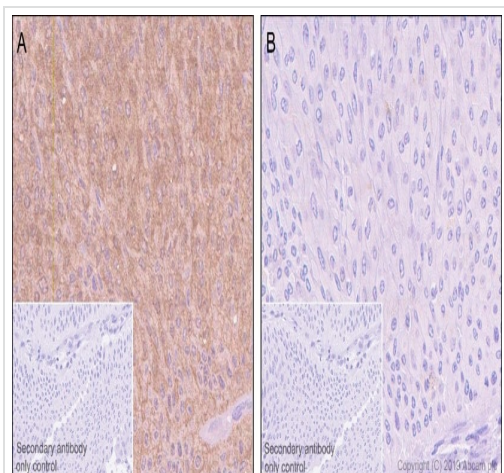
ELISA - Anti-PKC delta (phospho S645) antibody [EP1486Y] (ab108972)

Antigen PKCd S645Pho peptide, PKCd S645Pho unmodified peptide, Antigen concentration 1000ng/ml, Primary antibody concentration range 0~1000ng/ml, Secondary antibody Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L), Secondary antibody concentration 1:2500 dilution



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC delta (phospho S645) antibody [EP1486Y] (ab108972)

Immunohistochemical analysis of paraffin-embedded Human spleen carcinoma labeling PKC delta with ab108972 at 1/500 dilution (0.60 µg/ml). Positive staining on human spleen carcinoma without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab108972 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.



Immunohistochemical analysis of paraffin-embedded Human bladder carcinoma labeling PKC delta with ab108972 at 1/500 dilution (0.60 µg/ml). Positive staining on human bladder carcinoma without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab108972 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC delta (phospho S645) antibody [EP1486Y] (ab108972)

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