abcam

Product datasheet

Anti-PKC alpha (phospho T497) antibody [EP2608Y] ab76016

Recombinant RabMAb

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Overview

Product name Anti-PKC alpha (phospho T497) antibody [EP2608Y]

Description Rabbit monoclonal [EP2608Y] to PKC alpha (phospho T497)

Host species Rabbit

Tested applications Suitable for: Dot blot, WB, IHC-P

Unsuitable for: Flow Cyt or ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa, NIH/3T3, and C6 whole cell lysates. IHC-P: Human glioma, Mouse kidney, and Rat

kidney tissues.

General notes This product has switched from a hybridoma to recombinant production method on 2nd August

2023

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 -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

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Clonality Monoclonal
Clone number EP2608Y
Isotype IqG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab76016 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
Dot blot		1/1000.
WB		1/100000. Predicted molecular weight: 77 kDa.
IHC-P		1/10000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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

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Function This is a calcium-activated, phospholipid-dependent, serine- and threonine-specific enzyme. May

play a role in cell motility by phosphorylating CSPG4.

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.

Sequence similaritiesBelongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. PKC subfamily.

Contains 1 AGC-kinase C-terminal domain.

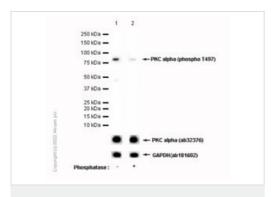
Contains 1 C2 domain.

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

Contains 1 protein kinase domain.

Cellular localization Cytoplasm. Cell membrane. Nucleus.

Images



Western blot - Anti-PKC alpha (phospho T497) antibody [EP2608Y] (ab76016)

All lanes : Anti-PKC alpha (phospho T497) antibody [EP2608Y] (ab76016) at 1/10000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate, then the membrane was incubated with Alkaline phosphatase

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 77 kDa **Observed band size:** 77 kDa

Exposure time: 10 seconds

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

All lanes : Anti-PKC alpha (phospho T497) antibody [EP2608Y] (ab76016) at 1/10000 dilution

Lane 1: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate **Lane 2**: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate,
then the membrane was incubated with Alkaline phosphatase

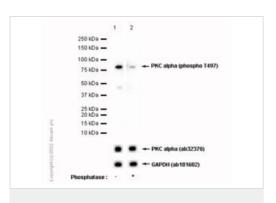
Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 77 kDa **Observed band size:** 77 kDa



Western blot - Anti-PKC alpha (phospho T497) antibody [EP2608Y] (ab76016)

Exposure time: 10 seconds

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

All lanes : Anti-PKC alpha (phospho T497) antibody [EP2608Y] (ab76016) at 1/10000 dilution

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

Lane 2: C6 (Rat glial tumor glial cell) whole cell lysate, then the membrane was incubated with Alkaline phosphatase

Lysates/proteins at 15 µg per lane.



All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 77 kDa **Observed band size:** 77 kDa

Exposure time: 5 seconds

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

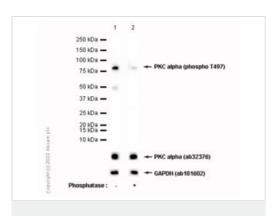
Dot blot analysis of PKC alpha (pT497) using ab76016 at 1:1000 (1.34 ug/ml) followed by a Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (ab97051) at 1:20000 dilution.

Lane 1: PKC alpha non-phospho peptide.

Lane 2: PKC alpha (phospho T497).

Blocking and dilution buffer: 5% NFDM/TBST.

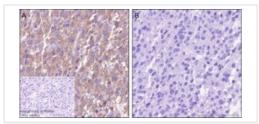
Exposure time: 10 seconds.



Western blot - Anti-PKC alpha (phospho T497) antibody [EP2608Y] (ab76016)

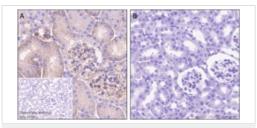


Dot Blot - Anti-PKC alpha (phospho T497) antibody [EP2608Y] (ab76016)



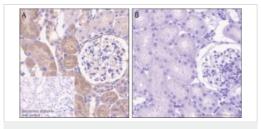
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC alpha (phospho T497) antibody [EP2608Y] (ab76016)

Immunohistochemical analysis of paraffin-embedded Human glioma tissue labeling PKC alpha with ab76016 at 1/10000 (0.134 µg/ml), with or without Lambda phosphatase treatment, then followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human glioma. No signal was detected when tissue was treated with Lambda phosphatase. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 30 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC alpha (phospho T497) antibody [EP2608Y] (ab76016)

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labeling PKC alpha with ab76016 at 1/10000 (0.134 µg/ml), with or without Lambda phosphatase treatment, then followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on Mouse kidney. No signal was detected when tissue was treated with Lambda phosphatase. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 30 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC alpha (phospho T497) antibody [EP2608Y] (ab76016)

Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labeling PKC alpha with ab76016 at 1/10000 (0.134 µg/ml), with or without Lambda phosphatase treatment, then followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on Rat kidney. No signal was detected when tissue was treated with Lambda phosphatase. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 30 mins.

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