abcam

Product datasheet

Anti-PKC alpha antibody [Y124] ab32376

KO VALIDATED Recombinant RabMAb

★★★★★ 13 Abreviews 106 References 20 Images

Overview

Product name	Anti-PKC alpha antibody [Y124]
Description	Rabbit monoclonal [Y124] to PKC alpha
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, IP, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human, Pig **Predicted to work with:** Goldfish
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK293, K562, HeLa, C6 cell lysates and pig skeletal muscle tissue lysates. IHC-P: Human lung carcinoma and human endometrium carcinoma tissues. ICC/IF: PMA-Treated and untreated wild-type HAP1 and HeLa cells.
General notes	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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	Y124
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32376 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
Flow Cyt (Intra)		1/20. For unpurified use at 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ **(6)**	1/1000 - 1/10000. Detects a band of approximately 75 kDa (predicted molecular weight: 77 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. **For unpurified use at 1/250 - 1/500.**
IP		1/20. **For unpurified use at 1/100.**
ICC/IF	★★★★★ **(2)**	1/200. Treatment with 200nM PMA for 30 minutes induces translocation of PKC alpha to the membrane.

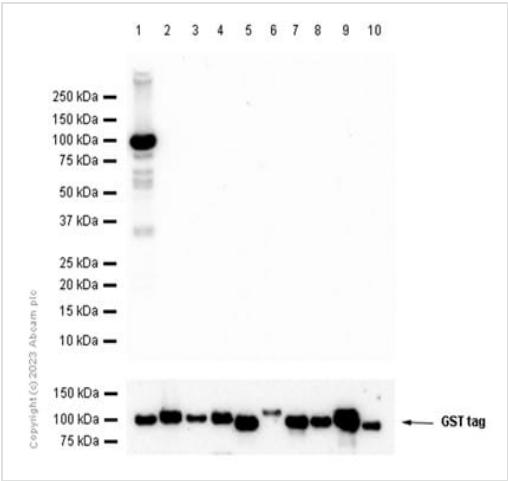
Target

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 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.

Cellular localization Cytoplasm. Cell membrane. Nucleus.

Images



Western blot - Anti-PKC alpha antibody [Y124] (ab32376)

All lanes : Anti-PKC alpha antibody [Y124] (ab32376) at 1/2000 dilution

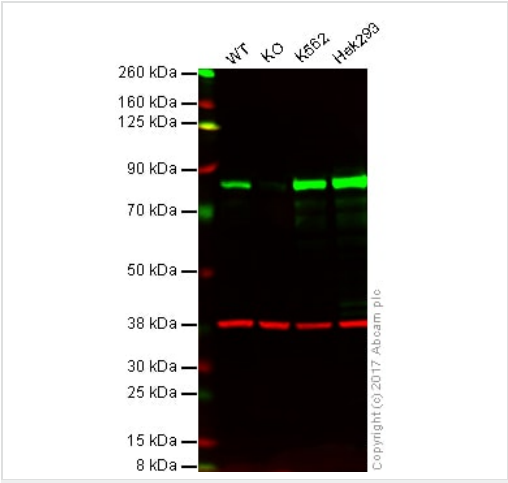
Lane 1 : Recombinant human PKC alpha protein (Active) (**ab55672**)
Lane 2 : Recombinant human PKC beta 1 protein (**ab60840**)
Lane 3 : Recombinant human PKC beta 2 protein (**ab60841**)
Lane 4 : Recombinant human PKC gamma protein (**ab60842**)
Lane 5 : Recombinant human PKC delta protein (**ab60844**)
Lane 6 : Recombinant human PKC epsilon protein (**ab60847**)
Lane 7 : Recombinant human PKC zeta protein (**ab60848**)
Lane 8 : Recombinant human PKC eta protein (**ab60849**)
Lane 9 : Recombinant human PKC theta/PRKCQ protein (**ab56641**)
Lane 10 : Recombinant human PKC iota protein (**ab60850**)

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

Predicted band size: 77 kDa
Observed band size: 105 kDa

Exposure time: 20 seconds

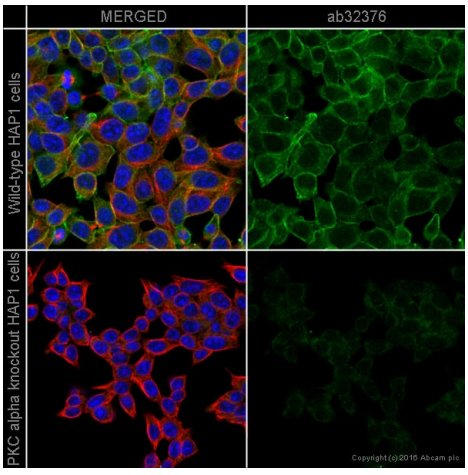
Blocking and diluting buffer and concentration: 5% NFDM/TBST.



Western blot - Anti-PKC alpha antibody [Y124] (ab32376)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: PKC alpha knockout HAP1 cell lysate (20 µg)
Lane 3: K562 cell lysate (20 µg)
Lane 4: HEK293 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab32376 observed at 77 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32376 was shown to specifically react with PKC alpha in wild-type HAP1 cells. No band was observed when PKC alpha knockout samples were examined. Wild-type and PKC alpha knockout samples were subjected to SDS-PAGE. ab32376 and **ab8245** (loading control to GAPDH) were diluted 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 hour at room temperature before imaging.

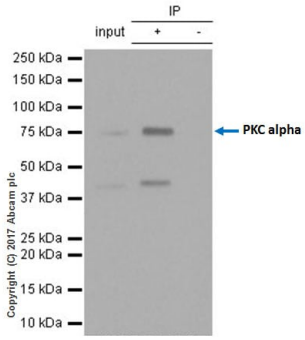


Immunocytochemistry/ Immunofluorescence - Anti-PKC alpha antibody [Y124] (ab32376)

Unpurified ab32376 staining PKCα in 200nM PMA-treated wild-type HAP1 cells (top panel) and PKCα in 200nM PMA-treated knockout HAP1 cells (bottom panel). The cells were treated with 200nM PMA for 30 minutes to induce translocation of PKCα to the cell membrane. The cells were then fixed with 100% methanol (5min), 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 ab32376 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 4% formaldehyde (10 min).

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



Immunoprecipitation - Anti-PKC alpha antibody [Y124] (ab32376)

ab32376 (purified) at 1:20 dilution (0.5ug) immunoprecipitating PKC alpha in 293 (Human embryonic kidney epithelial cell) whole cell lysate.

Lane 1 (input): 293 (Human embryonic kidney epithelial cell) whole cell lysate 10ug

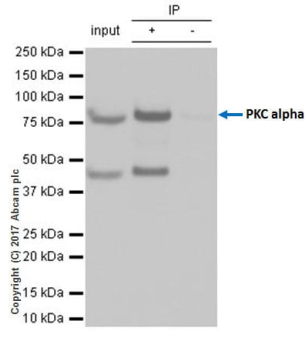
Lane 2 (+): ab32376 & 293 (Human embryonic kidney epithelial cell) whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32376 in 293 (Human embryonic kidney epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.

The band between 37kDa and 50kDa might be the C-term fragment. (PMID:10381525)



Immunoprecipitation - Anti-PKC alpha antibody [Y124] (ab32376)

ab32376 (purified) at 1:20 dilution (0.5ug) immunoprecipitating PKC alpha in Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate.

Lane 1 (input): Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10ug

Lane 2 (+): ab32376 & Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate

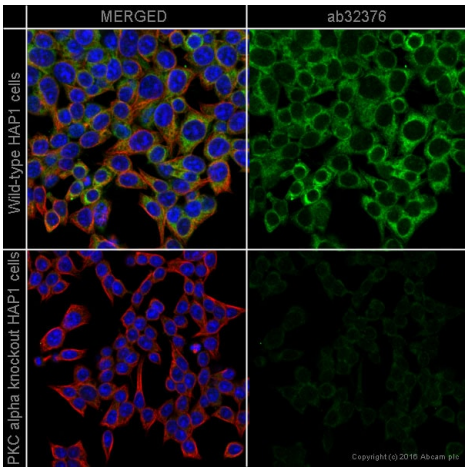
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32376 in Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.

The band between 37kDa and 50kDa might be the C-term

fragment. (PMID:10381525)

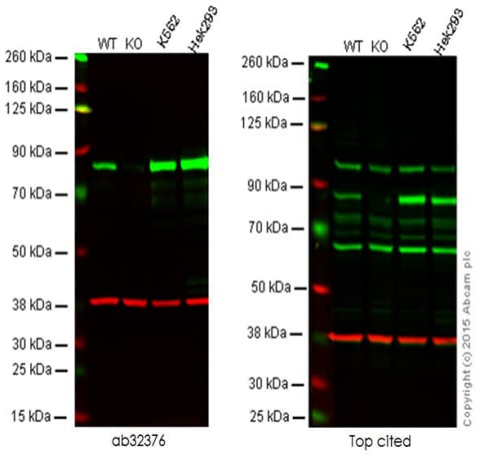


Immunocytochemistry/ Immunofluorescence - Anti-PKC alpha antibody [Y124] (ab32376)

Unpurified ab32376 staining PKCα in wild-type HAP1 cells (top panel) and PKCα in knockout HAP1 cells (bottom panel). In untreated conditions, PKCα is expressed in the cytoplasm of the cells. The cells were then fixed with 100% methanol (5min), 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 ab32376 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 4% formaldehyde (10 min).

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

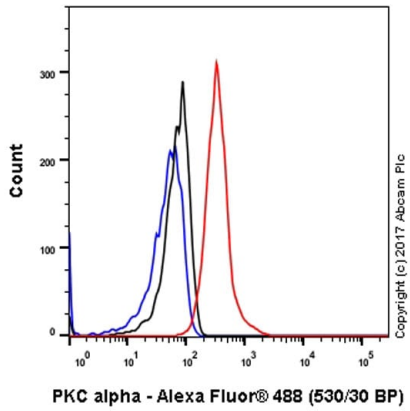


Western blot - Anti-PKC alpha antibody [Y124] (ab32376)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: PKC alpha knockout HAP1 cell lysate (20 µg)
Lane 3: K562 cell lysate (20 µg)
Lane 4: HEK293 cell lysate (20 µg)

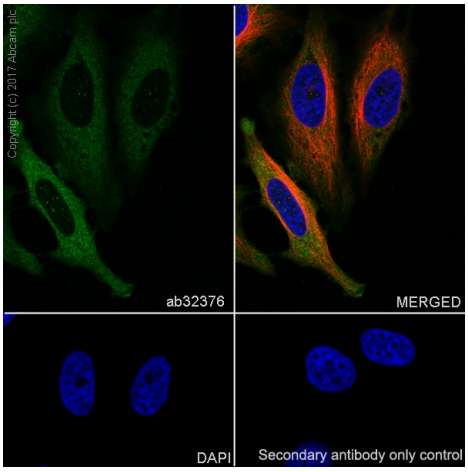
Lanes 1 - 4: Merged signal (red and green). Green - unpurified ab32376 observed at 77 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This western blot image is a comparison between ab32376 and a competitor's top cited rabbit polyclonal antibody.



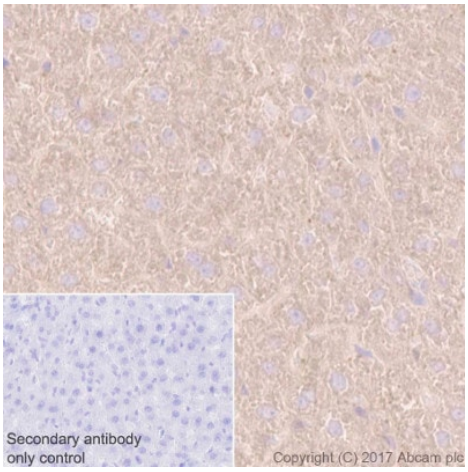
Flow Cytometry (Intracellular) - Anti-PKC alpha antibody [Y124] (ab32376)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling PKC alpha with purified ab32376 at 1/20 dilution (5 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluorr® 488) secondary antibody was used at 1/2000 dilution. Isotype control - 90% methanol. Unlabeled control - Rabbit monoclonal IgG (Black). Cell without incubation with primary antibody and secondary antibody (Blue).



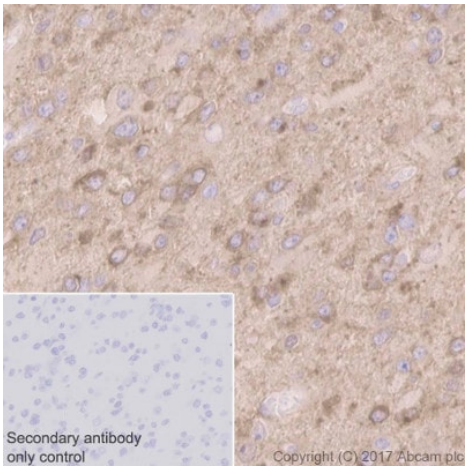
Immunocytochemistry/ Immunofluorescence - Anti-PKC alpha antibody [Y124] (ab32376)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling PKC alpha with Purified ab32376 at 1:250 dilution (0.4µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



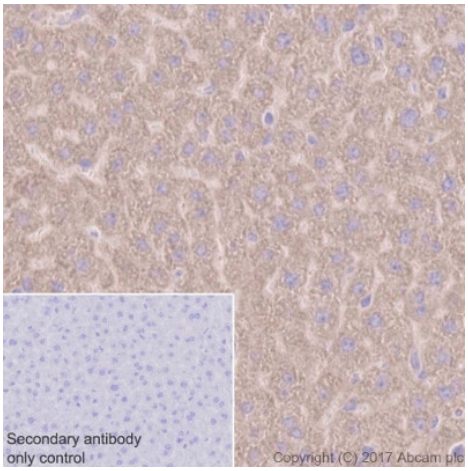
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC alpha antibody [Y124] (ab32376)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat liver tissue sections labeling PKC alpha with purified ab32376 at 1:100 dilution (1.01 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, pH9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



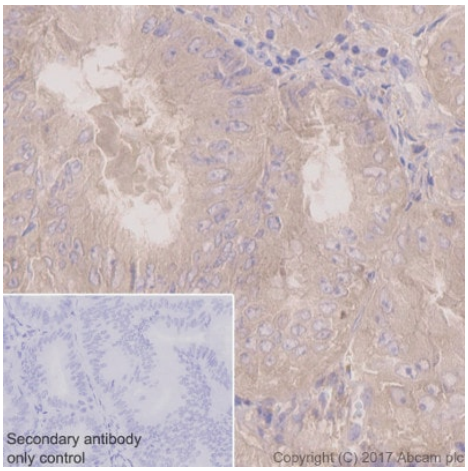
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC alpha antibody [Y124] (ab32376)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human glioma tissue sections labeling PKC alpha with purified ab32376 at 1:100 dilution (1.01 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, pH9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC alpha antibody [Y124] (ab32376)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse liver tissue sections labeling PKC alpha with purified ab32376 at 1:100 dilution (1.01 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, pH9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC alpha antibody [Y124] (ab32376)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human endometrium carcinoma tissue sections labeling PKC alpha with purified ab32376 at 1:100 dilution (1.01 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, pH9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

Western blot - Anti-PKC alpha antibody [Y124] (ab32376)

All lanes : Anti-PKC alpha antibody [Y124] (ab32376) at 1/5000 dilution (purified)

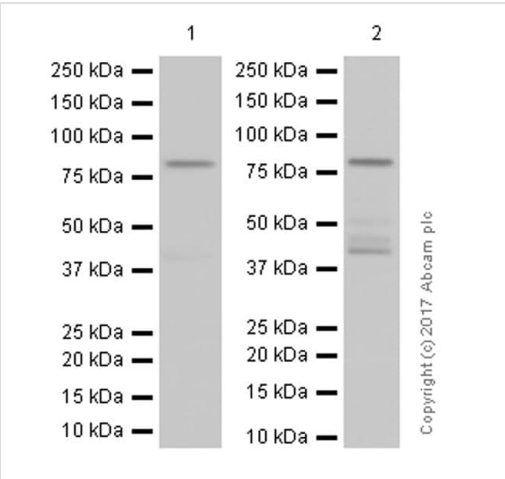
Lane 1 : 293 (Human embryonic kidney epithelial cell) whole cell lysates
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 77 kDa

Blocking and diluting buffer: 5% NFDM/TBST



Western blot - Anti-PKC alpha antibody [Y124] (ab32376)

All lanes : Anti-PKC alpha antibody [Y124] (ab32376) at 1/5000 dilution (purified)

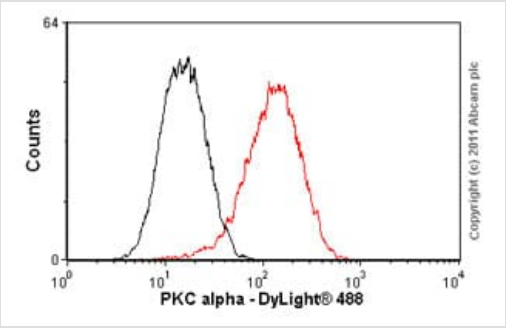
Lane 1 : C6 (Rat glial tumor glial cell) whole cell lysates
Lane 2 : Pig skeletal muscle lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

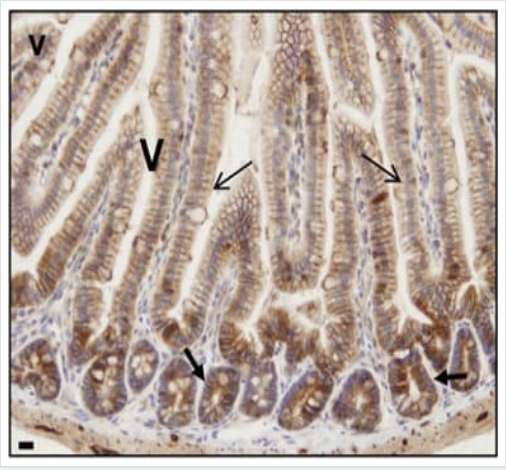
Predicted band size: 77 kDa

Blocking and diluting buffer: 5% NFDM/TBST



Flow Cytometry (Intracellular) - Anti-PKC alpha antibody [Y124] (ab32376)

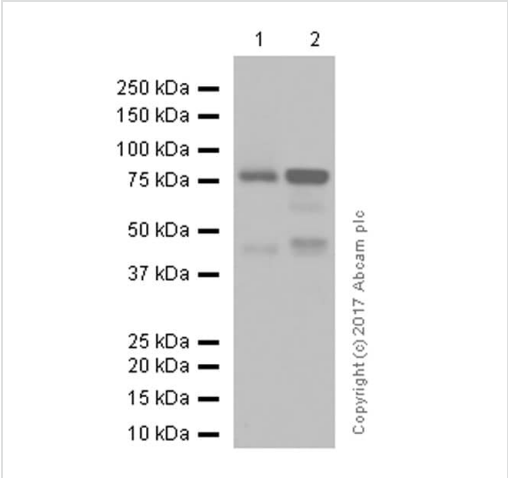
Overlay histogram showing HeLa cells stained with unpurified ab32376 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32376, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal IgG (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with methanol (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC alpha antibody [Y124] (ab32376)

Image from Hao F et al., J Biol Chem. 2011 May 20;286(20):18104-17. Epub 2011 Mar 18. Fig 1.; doi: 10.1074/jbc.M110.208488; May 20, 2011 The Journal of Biological Chemistry, 286, 18104-18117.

Immunohistochemical analysis of mouse small intestine tissue, staining PKC alpha with unpurified ab32376.



Western blot - Anti-PKC alpha antibody [Y124] (ab32376)

All lanes : Anti-PKC alpha antibody [Y124] (ab32376) at 1/10000 dilution (purified)

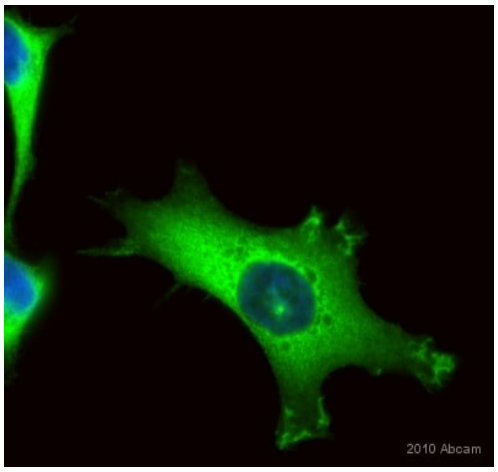
Lane 1 : K562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysates
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 77 kDa
Observed band size: 80 kDa

Blocking and diluting buffer: 5% NFDM/TBST
The band between 37kDa and 50kDa might be the C-term fragment. (PMID:10381525)



Immunocytochemistry/ Immunofluorescence - Anti-PKC alpha antibody [Y124] (ab32376)

This image is courtesy of an anonymous Abreview

ab32376 staining PKC in the HT1080 Cell line by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody (1/400 in PBS) for 1 hour at 22°C. An Alexa Fluor® 488-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody (1/1000).

Anti-PKC alpha antibody [Y124] (ab32376)

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