abcam

Product datasheet

Anti-PKC alpha antibody [Y124] - BSA and Azide free ab221611

KO 評価済 リコンビナント RabMAb

28 References 画像数 16

製品の概要

製品名	Anti-PKC alpha antibody [Y124] - BSA and Azide free
製品の詳細	Rabbit monoclonal [Y124] to PKC alpha - BSA and Azide free
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
種交差性	**交差種:** Mouse, Rat, Human, Pig **交差が予測される動物種:** Goldfish
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: 293 cell lysate. IHC-P: Human lung carcinoma tissue. ICC/IF: HeLa and PMA-Treated and untreated wild-type HAP1 cells.
特記事項	ab221611 is the carrier-free version of **ab32376**. 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.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	pH: 7.20 Constituent: PBS

キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	Y124
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab221611の使用に適用されます

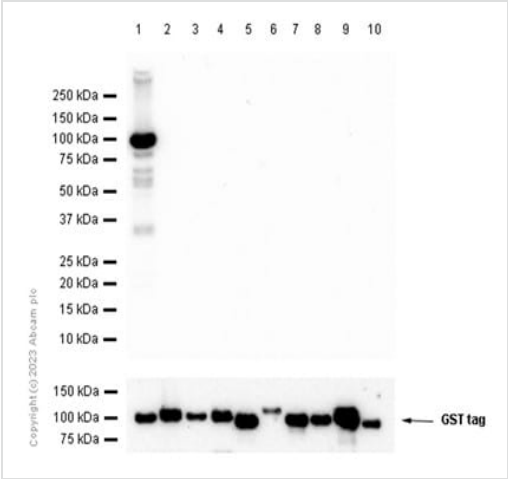
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 75 kDa (predicted molecular weight: 77 kDa).
IP		Use at an assay dependent concentration.
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 200nM PMA for 30 minutes induces translocation of PKC alpha to the membrane.

ターゲット情報

機能	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.
配列類似性	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.
細胞内局在	Cytoplasm. Cell membrane. Nucleus.

画像



Western blot - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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**)

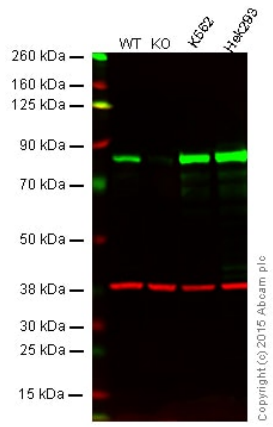
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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

Exposure time: 20 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).

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

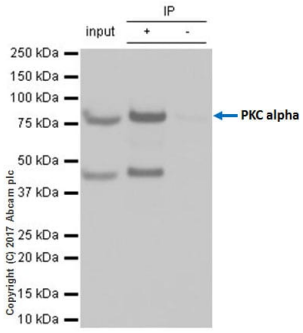


Western blot - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

This WB data was generated using the same anti-PKC alpha antibody clone, Y124, in a different buffer formulation (cat# **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.

Unpurified **ab32376** was shown to specifically react with PKC alpha when PKC alpha knockout samples were used. 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 h at room temperature before imaging.



Immunoprecipitation - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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

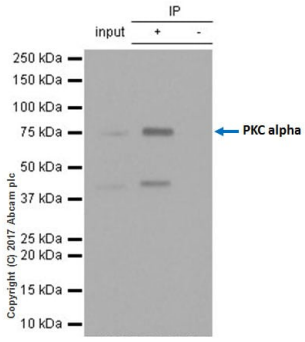
For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.

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

This data was developed using the same antibody clone in a

different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).



Immunoprecipitation - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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

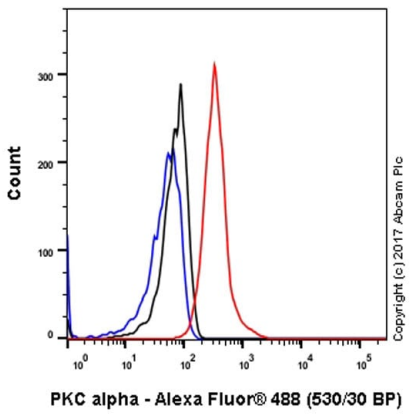
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab32376** in 293 (Human embryonic kidney epithelial cell) whole cell lysate

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)

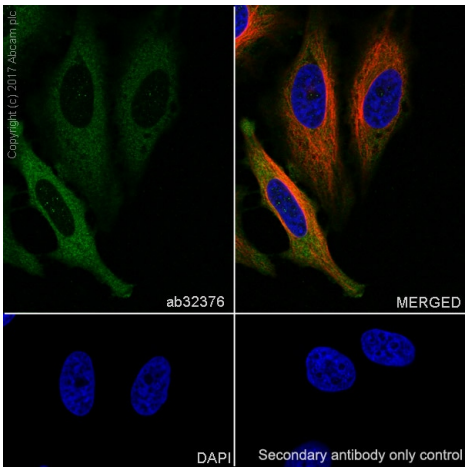
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).



Flow Cytometry (Intracellular) - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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 Fluor® 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).

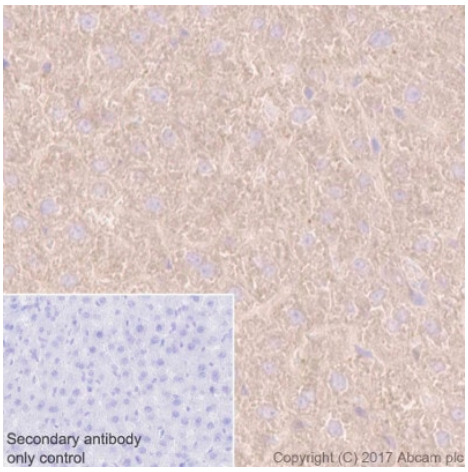
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).



Immunocytochemistry/ Immunofluorescence - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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.

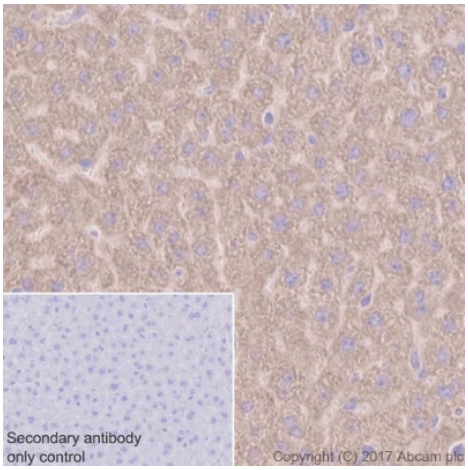
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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 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.

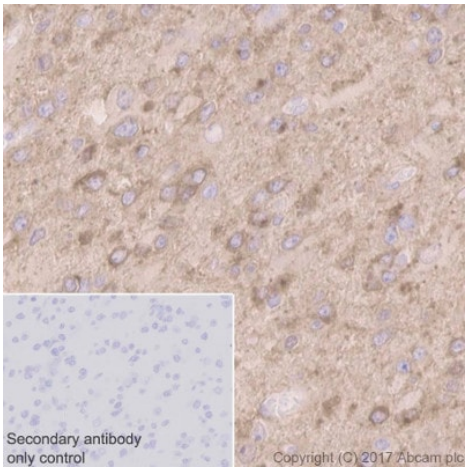
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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 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.

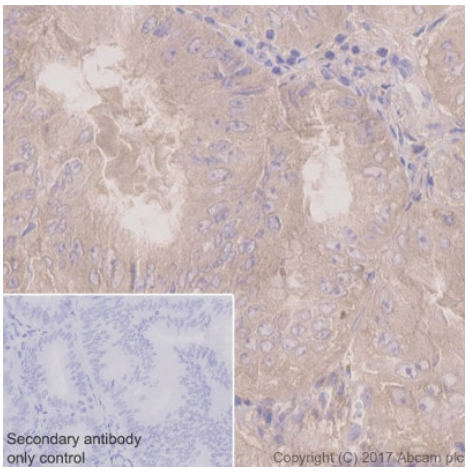
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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 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.

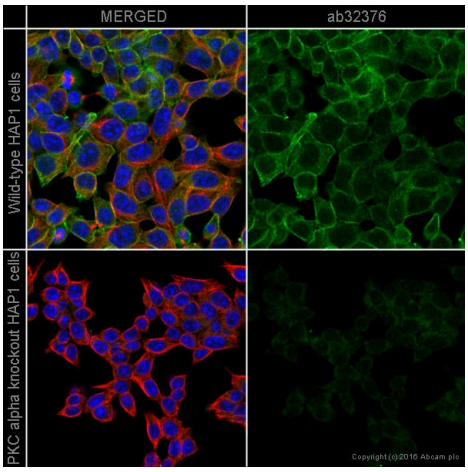
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).



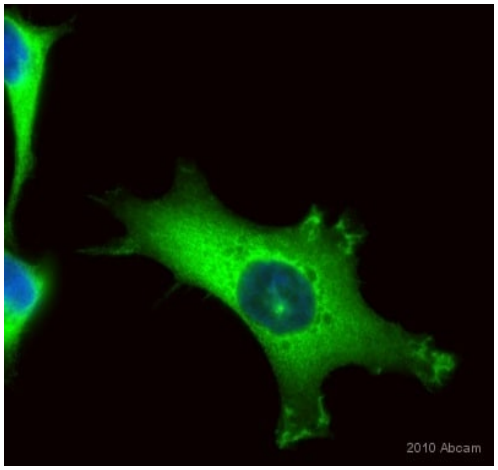
Immunocytochemistry/ Immunofluorescence - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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).

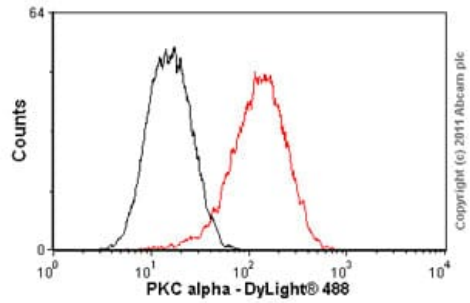
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).



Immunocytochemistry/ Immunofluorescence - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

This image is courtesy of an anonymous Abreview.

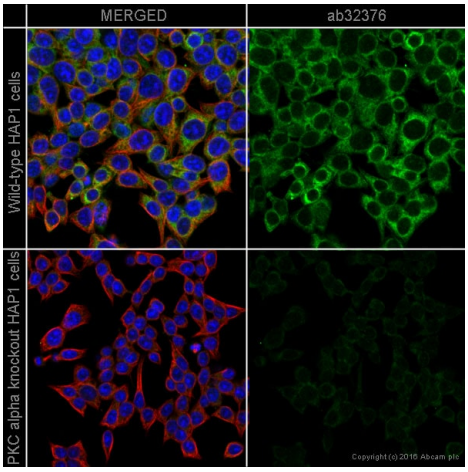
ab32376 staining PKC in the HT1080 Cell line by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody (1/400 in PBS) for 1 hour at 22°C. An Alexa Fluor® 488-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody (1/1000).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).



Flow Cytometry (Intracellular) - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32376**).



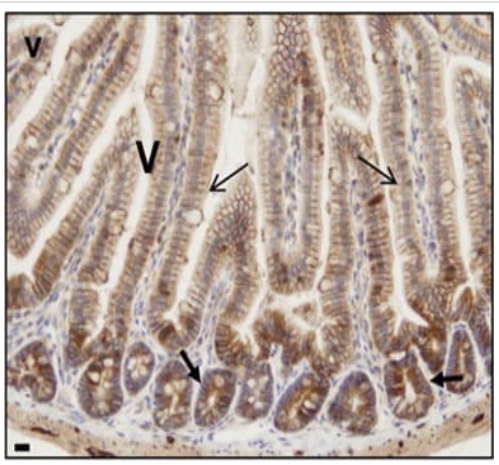
Immunocytochemistry/ Immunofluorescence - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

This ICC/IF data was generated using the same anti-PKC alpha antibody clone, Y124, in a different buffer formulation (cat# **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).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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.

This IHC data was generated using the same anti-PKC alpha antibody clone, Y124, in a different buffer formulation (cat# **ab32376**).

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

Anti-PKC alpha antibody [Y124] - BSA and Azide free (ab221611)

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