abcam

Product datasheet

Anti-PKC beta 2 antibody [Y125] ab32026

Recombinant RabMAb

★★★★★ 6 Abreviews 24 References

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Overview

Product name Anti-PKC beta 2 antibody [Y125]

Description Rabbit monoclonal [Y125] to PKC beta 2

Host species Rahhit

Specificity This antibody recognises PKC beta 2, it does not cross react with human PKC beta 1.

Immunogen's homology with PKC alpha is 77.8%. Cross-reactivity with PKC alpha has not been

tested.

Tested applications Suitable for: WB, IHC-P, ICC/IF, Flow Cyt (Intra)

Reacts with: Mouse, Rat, Human, Recombinant fragment Species reactivity

Predicted to work with: Cow, Pig

Immunogen Synthetic peptide within Human PKC beta 2 aa 600-700 (C terminal). The exact sequence is

proprietary.

Epitope ab32026 reacts with an epitope located in the region near the C terminus of PKC beta 2.

Positive control WB: K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate unboiled,

> Mouse brain lysate unboiled, Rat brain lysate unboiled, Active GST-tagged human PKC beta 2 full length protein. IHC-P: human gastric carcinoma, mouse cerebrum, rat cerebrum. ICC/IF: HeLa

(Human cervix adenocarcinoma epithelial cell) Flow cyto(intra): K-562 (Human chronic

myelogenous leukemia lymphoblast) 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 $\mathsf{RabMAb}^{\texttt{@}}$ 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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

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Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number Y125

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab32026 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
WB	**** <u>(4)</u>	1/1000 - 1/10000. Detects a band of approximately 77 kDa (predicted molecular weight: 77 kDa).
IHC-P	★★★★ <u>(1)</u>	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/250 - 1/500.
Flow Cyt (Intra)		1/50. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

Target

Function

Calcium-activated and phospholipid-dependent serine/threonine-protein kinase involved in various processes such as regulation of the B-cell receptor (BCR) signalosome, apoptosis and transcription regulation. Plays a key role in B-cell activation and function by regulating BCR-induced NF-kappa-B activation and B-cell suvival. Required for recruitment and activation of the IKK kinase to lipid rafts and mediates phosphorylation of CARD11/CARMA1 at 'Ser-559', 'Ser-644' and 'Ser-652', leading to activate the NF-kappa-B signaling. Involved in apoptosis following oxidative damage: in case of oxidative conditions, specifically phosphorylates 'Ser-36' of isoform p66Shc of SHC1, leading to mitochondrial accumulation of p66Shc, where p66Shc acts as a reactive oxygen species producer. Acts as a coactivator of androgen receptor (ANDR)-dependent transcription, by being recruited to ANDR target genes and specifically mediating phosphorylation of 'Thr-6' of histone H3 (H3T6ph), a specific tag for epigenetic transcriptional activation that prevents demethylation of histone H3 'Lys-4' (H3K4me) by LSD1/KDM1A. Also involved in triglyceride homeostasis. Serves as the receptor for phorbol esters, a class of tumor promoters.

Sequence similarities

 $\label{eq:Belongs} \textbf{Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. PKC subfamily.}$

Contains 1 AGC-kinase C-terminal domain.

Contains 1 C2 domain.

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

Post-translational modifications

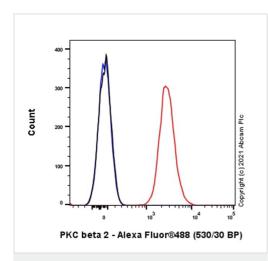
Contains 1 protein kinase domain.

Phosphorylation on Thr-500 within the activation loop renders it competent to autophosphorylate. Subsequent autophosphorylation of Thr-642 maintains catalytic competence, and autophosphorylation on Ser-661 appears to release the kinase into the cytosol. Autophosphorylation on other sites i.e. in the N-terminal and hinge regions have no effect on enzyme activity.

Cellular localization

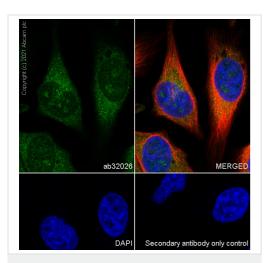
Cytoplasm. Nucleus. Membrane.

Images

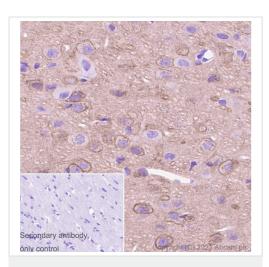


Flow Cytometry (Intracellular) - Anti-PKC beta 2 antibody [Y125] (ab32026)

Intracellular Flow Cytometry analysis of K-562 (Human chronic myelogenous leukemia lymphoblast) cells labeling PKC beta 2 with purified ab32026 at 1/20 dilution (5 ug/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor® 488, ab150081) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal lgG (Black) was used as a isotype control. Cell without incubation with primary antibody and secondary antibody (Blue) were used as unlabeled control.



Immunocytochemistry/ Immunofluorescence - Anti-PKC beta 2 antibody [Y125] (ab32026) Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling PKC beta 2 using ab32026. The cells were fixed with 4% paraformaldehyde then permeabilized with 0.1% Triton X-100. The cells were then incubated with ab32026 at 1:50 dilution followed by a further incubation with a Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 2 μg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI. Cells were counterstained using ab195889 Antialpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1:200 dilution (shown in red). Secondary antibody only control: PBS instead of the primary antibody.

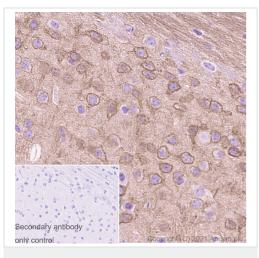


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC beta 2 antibody [Y125] (ab32026)

Immunohistochemical analysis of Paraffin-embedded sections rat cerebrum tissue labelling PKC beta 2 with ab32026 at 1/1000 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Staining on rat cerebrum tissue is observed. Counter stained with Haematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0).

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



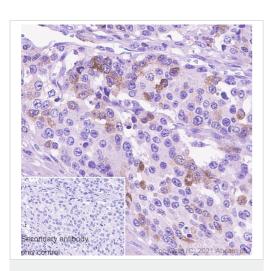
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC beta 2 antibody
[Y125] (ab32026)

Immunohistochemical analysis of Paraffin-embedded sections mouse cerebrum tissue labelling PKC beta 2 with ab32026 at 1/1000 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Staining on mouse cerebrum tissue is observed. Counter stained with Haematoxylin.

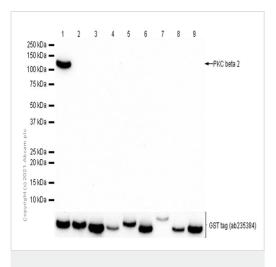
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval using Bond $^{\mbox{\tiny TM}}$ Epitope Retrieval Solution 2 (pH 9.0).

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC beta 2 antibody [Y125] (ab32026)



Western blot - Anti-PKC beta 2 antibody [Y125] (ab32026)

Immunohistochemical analysis of Paraffin-embedded sections human gastric carcinoma tissue labelling PKC beta 2 with ab32026 at 1/300 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Staining on human gastric carcinoma tissue is observed. Counter stained with Haematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0).

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

All lanes : Anti-PKC beta 2 antibody [Y125] (ab32026) at 1/1000 dilution

Lane 1: Recombinant human PKC beta 2 protein (ab60841)

Lane 2 : Recombinant human PKC beta 1 protein (ab60840)

Lane 3: Recombinant human PKC delta protein (ab60844)

Lane 4: Recombinant human PKC eta protein (ab60849)

Lane 5: Recombinant human PKC epsilon protein (ab60847)

Lane 6 : Recombinant human PKC theta/PRKCQ protein (ab56641)

Lane 7: Recombinant human PKC mu/PKD protein (ab60873)

Lane 8 : Recombinant human PKC alpha protein (Active)

(<u>ab55672</u>)

Lane 9: Recombinant human PKC gamma protein (ab60842)

Secondary

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

Predicted band size: 77 kDa

Observed band size: 100 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST. **ab235384** was used to detect GST tag.

All lanes : Anti-PKC beta 2 antibody [Y125] (ab32026) at 1/1000 dilution

Lane 1: K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate boiled

Lane 2: K-562 (Human chronic myelogenous leukemia

lymphoblast) whole cell lysate unboiled

Lane 3: Mouse brain lysate boiled

Lane 4: Mouse brain lysate unboiled

Lane 5 : Rat brain lysate boiled

Lane 6 : Rat brain lysate unboiled

Lysates/proteins at 20 µg per lane.

1 2 3 4 5 6

250 kDa —
150 kDa —
100 kDa —
75 kDa —

37 kDa —

37 kDa —

10 kDa —
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Western blot - Anti-PKC beta 2 antibody [Y125] (ab32026)

Secondary

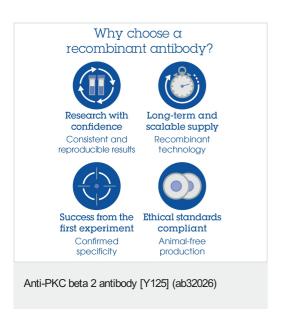
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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

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

ab181602 was used as GAPDH loading control.

We recommend not to boil samples when loading lysate.



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