## abcam

## Product datasheet

## Anti－PKC（phospho T514）antibody［EP2730Y］ab109539 ココンビナント RabMAb

## 13 References 画像数 13

## 製品の概要

| 製品名 | Anti－PKC（phospho T514）antibody［EP2730Y］ |
| :---: | :---: |
| 製品の詳細 | Rabbit monoclonal［EP2730Y］to PKC（phospho T514） |
| 由来種 | Rabbit |
| アプリケーション | 適用あり：Indirect ELISA，Dot blot，WB，IP，HCC－P |
| 種交差性 | 交差種：Mouse，Rat，Human |
| 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 | HeLa cell lysate；Human brain tissue；Mouse cerebellum；Rat brain；Human cerebral cortex． |
| 特記事項 | 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 ${ }^{\circledR}$ technology is a patented hybridoma－based technology for making rabbit |

## 製品の特性

| 製品の状態 | Liquid |
| :--- | :--- |
| 保存方法 | Shipped at $4^{\circ} \mathrm{C}$ ．Store at $+4^{\circ} \mathrm{C}$ short term（1－2 weeks）．Upon delivery aliquot．Store at $-20^{\circ} \mathrm{C}$ long |
|  | term．Avoid freeze／thaw cycle． |
| バッファー | $\mathrm{pH}: 7.20$ |
|  | Preservative： $0.01 \%$ Sodium azide |
|  | Constituents： $59 \%$ PBS， $40 \%$ Glycerol（glycerin，glycerine）， $0.5 \%$ BSA |
| 精製度 | Protein A purified |
| ポリ／モノ | モノクローナル |
| クローン名 | EP2730Y |
| アイソタイプ | lgG |

## 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ab109539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 |
| :--- | :--- | :--- |
| Indirect ELISA |  | Use at an assay dependent concentration． |
| Dot blot |  | Use at an assay dependent concentration． |
| WB |  | $1 / 1000-1 / 10000$. Predicted molecular weight： 78 kDa. |
| IP |  | $1 / 20$. For unpurified use at $\mathbf{1 / 5 0 .}$ <br> IHC－P |
|  |  | $1 / 300$. Perform heat mediated antigen retrieval with citrate buffer <br> pHer unpurified use at $\mathbf{1 / 1 0 0} \mathbf{- 1 / 2 5 0 .}$ |

## ターゲット情報

Calcium－activated，phospholipid－and diacylglycerol（DAG）－dependent serine／threonine－protein kinase that is involved in positive and negative regulation of cell proliferation，apoptosis， differentiation，migration and adhesion，tumorigenesis，cardiac hypertrophy，angiogenesis， platelet function and inflammation，by directly phosphorylating targets such as RAF1，BCL2， CSPG4，TNNT2／CTNT，or activating signaling cascade involving MAPK1／3（ERK1／2）and RAP1GAP．Involved in cell proliferation and cell growth arrest by positive and negative regulation of the cell cycle．Can promote cell growth by phosphorylating and activating RAF1，which mediates the activation of the MAPK／ERK signaling cascade，and／or by up－regulating CDKN1A， which facilitates active cyclin－dependent kinase（CDK）complex formation in glioma cells．In intestinal cells stimulated by the phorbol ester PMA，can trigger a cell cycle arrest program which is associated with the accumulation of the hyper－phosphorylated growth－suppressive form of RB1 and induction of the CDK inhibitors CDKN1A and CDKN1B．Exhibits anti－apoptotic function in glioma cells and protects them from apoptosis by suppressing the p53／TP53－mediated activation of IGFBP3，and in leukemia cells mediates anti－apoptotic action by phosphorylating BCL2．During macrophage differentiation induced by macrophage colony－stimulating factor（CSF1），is translocated to the nucleus and is associated with macrophage development．After wounding， translocates from focal contacts to lamellipodia and participates in the modulation of desmosomal adhesion．Plays a role in cell motility by phosphorylating CSPG4，which induces association of CSPG4 with extensive lamellipodia at the cell periphery and polarization of the cell accompanied by increases in cell motility．Is highly expressed in a number of cancer cells where it can act as a tumor promoter and is implicated in malignant phenotypes of several tumors such as gliomas and breast cancers．Negatively regulates myocardial contractility and positively regulates angiogenesis，platelet aggregation and thrombus formation in arteries．Mediates hypertrophic growth of neonatal cardiomyocytes，in part through a MAPK1／3（ERK1／2）－dependent signaling pathway，and upon PMA treatment，is required to induce cardiomyocyte hypertrophy up to heart failure and death，by increasing protein synthesis，protein－DNA ratio and cell surface area． Regulates cardiomyocyte function by phosphorylating cardiac troponin T（TNNT2／CTNT），which induces significant reduction in actomyosin ATPase activity，myofilament calcium sensitivity and myocardial contractility．In angiogenesis，is required for full endothelial cell migration，adhesion to vitronectin（VTN），and vascular endothelial growth factor A（VEGFA）－dependent regulation of
kinase activation and vascular tube formation．Involved in the stabilization of VEGFA mRNA at post－transcriptional level and mediates VEGFA－induced cell proliferation．In the regulation of calcium－induced platelet aggregation，mediates signals from the CD36／GP4 receptor for granule release，and activates the integrin heterodimer ITGA2B－ITGB3 through the RAP1GAP pathway for adhesion．During response to lipopolysaccharides（LPS），may regulate selective LPS－induced macrophage functions involved in host defense and inflammation．But in some inflammatory responses，may negatively regulate NF－kappa－B－induced genes，through IL1A－dependent induction of NF－kappa－B inhibitor alpha（NFKBIA／IKBA）．Upon stimulation with 12－O－ tetradecanoylphorbol－13－acetate（TPA），phosphorylates EIF4G1，which modulates EIF4G1 binding to MKNK1 and may be involved in the regulation of EIF4E phosphorylation． Phosphorylates KIT，leading to inhibition of KIT activity．Phosphorylates ATF2 which promotes cooperation between ATF2 and JUN，activating transcription．

配列類似性

細胞内局在 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． Cytoplasm．Cell membrane．Mitochondrion membrane．Nucleus．

画像


Western blot－Anti－PKC（phospho T514）antibody ［EP2730Y］（ab109539）

All lanes ：Anti－PKC（phospho T514）antibody［EP2730Y］ （ab109539）at 1／1000 dilution

Lane 1 ：Recombinant human PKC alpha protein（Active）
（ab55672）at $0.5 \mu \mathrm{~g}$
Lane 2 ：Recombinant human PKC beta 1 protein（ $\mathbf{( \mathbf { a b 6 0 8 4 0 } )}$ ）at 0.2 $\mu \mathrm{g}$
Lane 3 ：Recombinant human PKC beta 2 protein（ab60841）at 0.2 $\mu \mathrm{g}$

Lane 4 ：Recombinant human PKC delta protein（ab60844）at 0.05 $\mu \mathrm{g}$

Lane 5 ：Recombinant human PKC eta protein（ab60849）at $0.5 \mu \mathrm{~g}$
Lane 6 ：Recombinant human PKC epsilon protein（ $\mathbf{( a b 6 0 8 4 7 )}$ ）at 1
$\mu \mathrm{g}$
Lane 7 ：Recombinant human PKC theta／PRKCQ protein
（ab56641）at $0.2 \mu \mathrm{~g}$
Lane 8 ：Recombinant human PKC mu／PKD protein（ab60873）at $1 \mu \mathrm{~g}$
Lane 9 ：Recombinant human PKC gamma protein（ab60842）at $0.1 \mu \mathrm{~g}$

## Secondary

All lanes：Goat Anti－Rabbit lgG H\＆L（HRP）（ab97051）at 1／100000 dilution

Predicted band size: 78 kDa
Observed band size: 80 kDa

Exposure time: 180 seconds

Blocking and dilution buffer: 5\% NFDM/TBST.
ab109539 could detect PKC gamma (pT514), PKC alpha (pT497), PKC beta ( $p$ T500), PKC delta ( $p$ T507), PKC eta ( $p$ T513), PKC epsilon (pT566) and PKC theta (pT538).

Active human PKC alpha full length protein (Catalog\#ab55672) contains aa1-672 with GST-tag;

Active human PKC beta 1 full length protein (Catalog\#ab60840) contains aa1-671 with GST-tag;
Active human PKC beta 2 full length protein (Catalog\#ab60841) contains aa1-673 with GST-tag;
Active human PKC delta full length protein (Catalog\#ab60844) contains aa1-676 with GST-tag;
Active human PKC eta full length protein (Catalog\#ab60849) contains aa1-683 with GST-tag;
Active human PKC epsilon full length protein (Catalog\#ab60847) contains aa1-737 with GST-tag;
Active human PKC theta full length protein (Catalog\#ab56641) contains aa1-706 with GST-tag;
Active human PKC mu full length protein (Catalog\#ab60873)
contains aa1-912 with GST-tag;
Active human PKC gamma full length protein (Catalog\#ab60842)
contains aa1-697 with GST-tag.


Western blot - Anti-PKC (phospho T514) antibody [EP2730Y] (ab109539)

All lanes: Anti-PKC (phospho T514) antibody [EP2730Y] (ab109539) at 1/2000 dilution (purified)

Lane 1 : Rat brain lysates
Lane 2 : Rat brain lysates and then the membrane was incubated with phosphatase.

Lysates/proteins at $15 \mu \mathrm{~g}$ per lane.

## Secondary

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

Predicted band size: 78 kDa

Blocking and diluting buffer : 5\% NFDM/TBST

All lanes: Anti-PKC (phospho T514) antibody [EP2730Y]
(ab109539) at $1 / 2000$ dilution (purified)

Lane 1 : Mouse cerebellum lysates
Lane 2 : Mouse cerebellum lysates and then the membrane was incubated with phosphatase.

Lysates/proteins at $15 \mu \mathrm{~g}$ per lane.

## Secondary

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

Predicted band size: 78 kDa

Blocking and diluting buffer : $5 \%$ NFDM/TBST


All lanes: Anti-PKC (phospho T514) antibody [EP2730Y] (ab109539) at $1 / 5000$ dilution (purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) treated with Phorbol-12-myristate-13-acetate whole cell lysates Lane 3 : HeLa (Human cervix adenocarcinoma epithelial cell) treated with Phorbol-12-myristate-13-acetate whole cell lysates. The membrane was then incubated with phosphatase.

Lysates/proteins at $15 \mu \mathrm{~g}$ per lane.

## Secondary

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

Predicted band size: 78 kDa
Observed band size: 80 kDa

## Exposure time: 3 minutes

Blocking and dilution buffer: 5\% NFDM/TBST.

| KDa |  |  |  |
| :---: | :---: | :---: | :---: |
| 250- |  |  |  |
| 150- |  |  |  |
| 100- |  |  |  |
| 75- |  | - |  |
| 50- |  |  |  |
| 37- |  |  |  |
| 25- |  |  |  |
| 20- |  |  |  |
| 15- |  |  |  |
| 10- |  |  |  |
| PKC gamma ( p T514) peptide : | + | - | - |
| PKC gamma non-phospho peptide : | - | + | - |
| Control : | - | - | - |

Western blot - Anti-PKC (phospho T514) antibody [EP2730Y] (ab109539)


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC (phospho T514) antibody [EP2730Y] (ab109539)

All lanes: Anti-PKC (phospho T514) antibody [EP2730Y] (ab109539) at $0.02 \mu \mathrm{~g} / \mathrm{ml}$ (unpurified)

Lane 1: HeLa cell lysate with PKC gamma (pT514) peptide
Lane 2: HeLa cell lysate with PKC gamma non-phospho peptide
Lane 3 : HeLa cell lysate

Predicted band size: 78 kDa
Observed band size: 80 kDa

Exposure time: 15 seconds

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat cerebellum tissue sections labeling PKC with Purified ab109539 at 1:300 dilution $(1.14 \mu \mathrm{~g} / \mathrm{ml})$. Heat mediated antigen retrieval was performed using citrate buffer, pH 6.0. 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 paraffinembedded sections) - Anti-PKC (phospho T514) antibody [EP2730Y] (ab109539)


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse cerebellum tissue sections labeling PKC with Purified ab109539 at 1:300 dilution $(1.14 \mu \mathrm{~g} / \mathrm{ml})$. Heat mediated antigen retrieval was performed using citrate buffer, pH 6.0. 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) analysis of Human cerebral cortex tissue sections labeling PKC with Purified ab109539 at 1:300 dilution ( $1.14 \mu \mathrm{~g} / \mathrm{ml}$ ). Heat mediated antigen retrieval was performed using citrate buffer, pH 6.0. 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.

Unpurified ab109539 at 1/100 dilution staining PKC (phospho T514) in paraffin-embedded Human brain tissue by Immunohistochemistry.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

ab109539 (purified) at 1:20 dilution $(2 \mu \mathrm{~g})$ immunoprecipitating PKC in Rat brain lysate.
Lane 1 (input): Rat brain lysate $10 \mu \mathrm{~g}$
Lane 2 (+): ab109539 \& Rat brain lysate
Lane 3 (-): Rabbit monoclonal lgG (ab172730) instead of ab109539 in Rat brain 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.

Various phosphorylated PKC peptides at $1000 \mathrm{ng} / \mathrm{ml}$ stained for PKC (phospho T514) using ab109539 at 0-1000 ng/ml in ELISA. Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG $(\mathrm{H}+\mathrm{L})$ was used as a secondary antibody at a concentration of 1/2500.

Antigen:
Human PKC gamma (pT514)
Human PKC alpha (pT497)
Human PKC beta (pT500)
Human PKC delta (pT507)
Human PKC eta (pT513)
Human PKC epsilon (pT566)
Human PKC theta ( p T538)
Human PKC gamma non-phospho


Dot Blot - Anti-PKC (phospho T514) antibody [EP2730Y] (ab109539)

Various phosphorylated PKC peptides stained for PKC (phospho T514) using ab109539 at $0.543 \mu \mathrm{~g} / \mathrm{ml}$ in Dot blot. Goat Anti-Rabbit lgG H\&L (HRP) (ab97051) was used as the secondary antibody at a dilution of 1/100,000. Exposure time: 180 seconds.

Blocking and dilution buffer: 5\% NFDM/TBST.
Lane 1: Human PKC gamma (pT514) peptide
Lane 2: Human PKC alpha (pT497) peptide
Lane 3: Human PKC gamma non-phospho peptide
Lane 4: Human PKC beta ( p T500) peptide
Lane 5: Human PKC delta ( $\mathrm{pT507} \mathrm{)} \mathrm{peptide}$
Lane 6: Human PKC eta ( $\mathrm{p} T 513$ ) peptide
Lane 7: Human PKC epsilon (pT566) peptide
Lane 8: Human PKC theta ( $\mathrm{p} T 538$ ) peptide
ab109539 could detect PKC gamma (pT514), PKC alpha (pT497), PKC beta (pT500), PKC delta (pT507), PKC eta (pT513), PKC epsilon (pT566) and PKC theta (pT538).
$\left.\begin{array}{c}\text { Why choose a } \\ \text { recombinont antibody? } \\ \begin{array}{c}\text { Success from the } \\ \text { confidence } \\ \text { Consistent and } \\ \text { reproducible results }\end{array} \\ \begin{array}{c}\text { Long-term and } \\ \text { scalable supply } \\ \text { Recombinant } \\ \text { technology }\end{array} \\ \text { Conferimed standards } \\ \text { specificity }\end{array} \quad \begin{array}{l}\text { Animal-free } \\ \text { production }\end{array}\right]$

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