abcam

Product datasheet

Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free ab247898

Recombinant RabMAb

13 Images

Overview

Product name	Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free
Description	Rabbit monoclonal [EP2730Y] to PKC (phospho T514) - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, Indirect ELISA, IP, Dot blot, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
General notes	ab247898 is the carrier-free version of **ab109539**. 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. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP2730Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab247898 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
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Indirect ELISA		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Dot blot		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 78 kDa.

Target

Function 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.

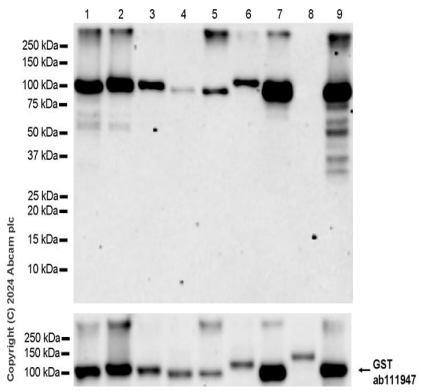
Sequence similarities

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. Mitochondrion membrane. Nucleus.

Images



Western blot - Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

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 µg
Lane 2 : Recombinant human PKC beta 1 protein (**ab60840**) at 0.2 µg
Lane 3 : Recombinant human PKC beta 2 protein (**ab60841**) at 0.2 µg
Lane 4 : Recombinant human PKC delta protein (**ab60844**) at 0.05 µg
Lane 5 : 60849 at 0.5 µg
Lane 6 : Recombinant human PKC epsilon protein (**ab60847**) at 1 µg
Lane 7 : Recombinant human PKC theta/PRKCQ protein (**ab56641**) at 0.2 µg
Lane 8 : Recombinant human PKC mu/PKD protein (**ab60873**) at 1 µg
Lane 9 : Recombinant human PKC gamma protein (**ab60842**) at 0.1 µg

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 180 seconds

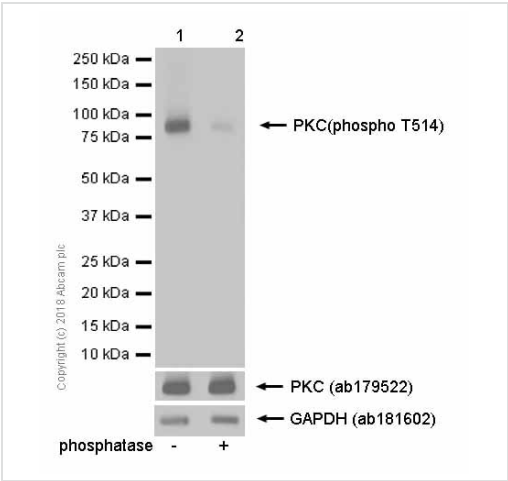
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109539**).

Blocking and dilution buffer: 5% NFDM/TBST.

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).

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] - BSA and Azide free (ab247898)

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.

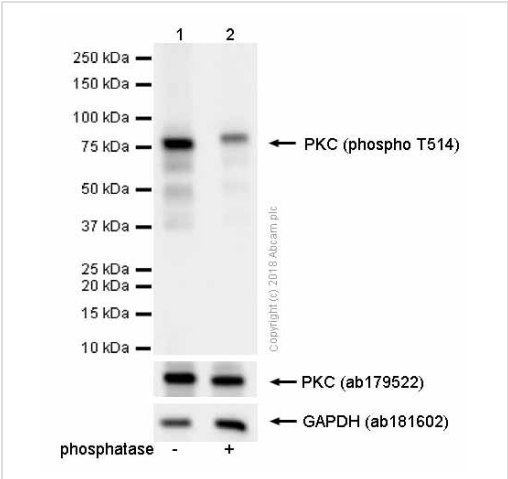
Lysates/proteins at 15 µg per lane.

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

Predicted band size: 78 kDa

This data was developed using **ab109539**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

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.

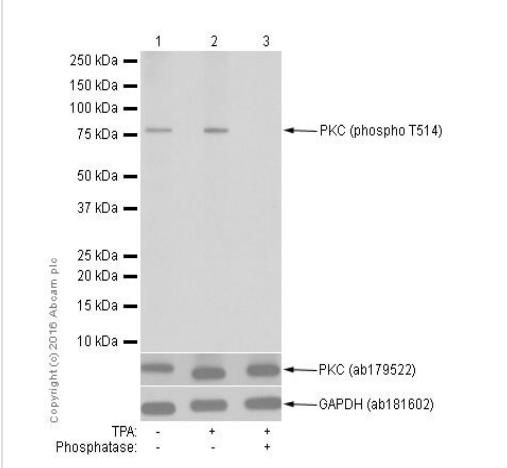
Lysates/proteins at 15 µg per lane.

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

Predicted band size: 78 kDa

This data was developed using **ab109539**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

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 µg per lane.

Secondary

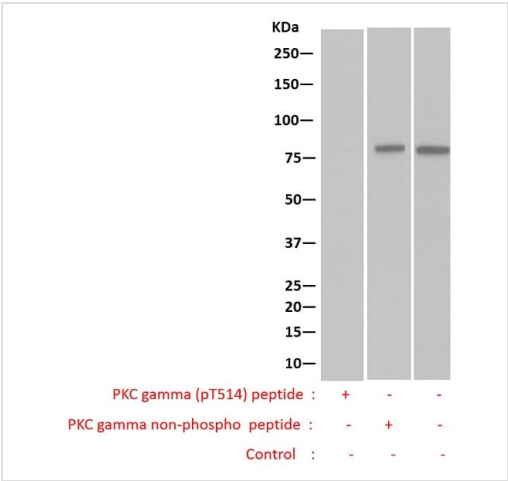
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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

Exposure time: 3 minutes

This data was developed using **ab109539**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

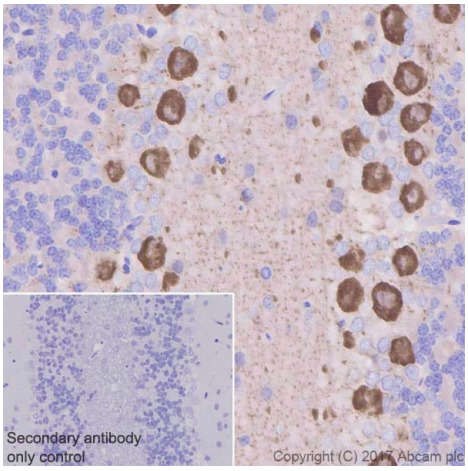
All lanes : Anti-PKC (phospho T514) antibody [EP2730Y] (**ab109539**) at 0.02 µg/ml (unpurified)

All lanes : HeLa cell lysate

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

Exposure time: 15 seconds

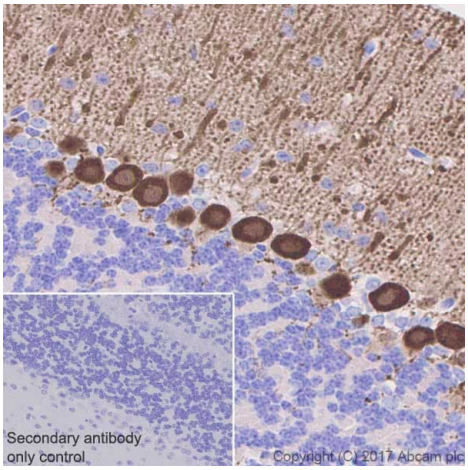
This data was developed using **ab109539**, the same antibody clone in a different buffer formulation.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

This data was developed using **ab109539**, the same antibody clone in a different buffer formulation.

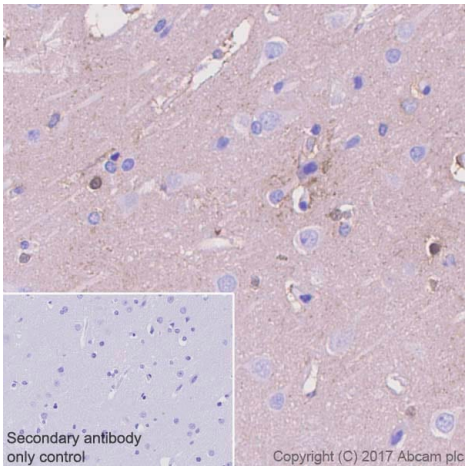
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat cerebellum tissue sections labeling PKC with Purified **ab109539** at 1:300 dilution (1.14 µg/ml). Heat mediated antigen retrieval was performed using citrate buffer, pH 6.0. Tissue was counterstained with Hematoxylin. 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) - Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

This data was developed using **ab109539**, the same antibody clone in a different buffer formulation.

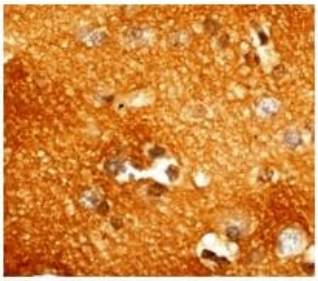
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse cerebellum tissue sections labeling PKC with Purified **ab109539** at 1:300 dilution (1.14 µg/ml). Heat mediated antigen retrieval was performed using citrate buffer, pH 6.0. Tissue was counterstained with Hematoxylin. 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) - Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

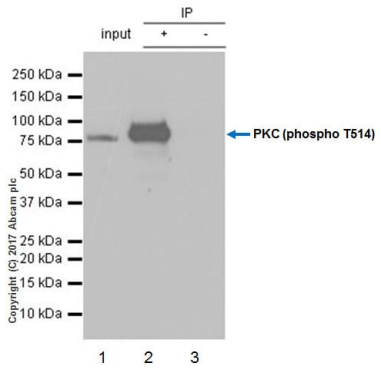
This data was developed using **ab109539**, the same antibody clone in a different buffer formulation.

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 µg/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) - Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

This data was developed using **ab109539**, the same antibody clone in a different buffer formulation.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.



Immunoprecipitation - Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

This data was developed using **ab109539**, the same antibody clone in a different buffer formulation.

ab109539 (purified) at 1:20 dilution (2µg) immunoprecipitating PKC in Rat brain lysate.

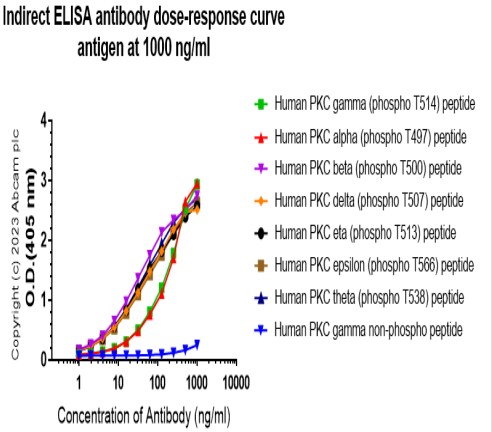
Lane 1 (input): Rat brain lysate 10µg

Lane 2 (+): **ab109539** & Rat brain lysate

Lane 3 (-): Rabbit monoclonal IgG (**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.



Indirect ELISA - Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

Various phosphorylated PKC peptides at 1000 ng/ml stained for PKC (phospho T514) using **ab109539** at 0-1000 ng/ml in ELISA.

Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+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)

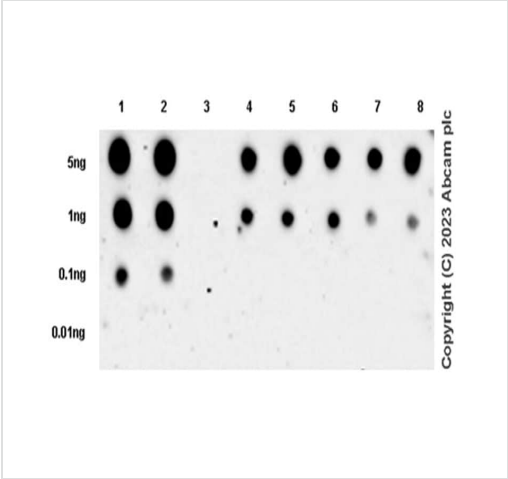
Human PKC eta (pT513)

Human PKC epsilon (pT566)

Human PKC theta (pT538)

Human PKC gamma non-phospho

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109539**)



Dot Blot - Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

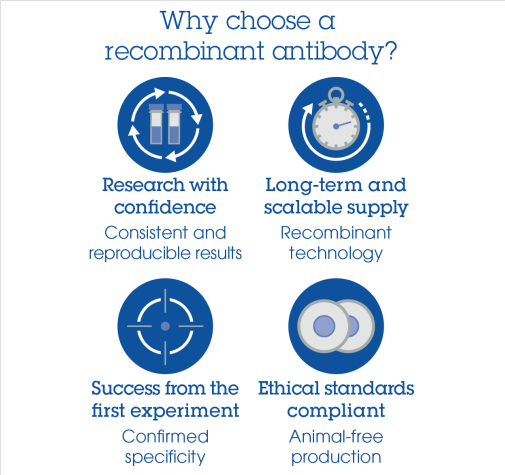
Various phosphorylated PKC peptides stained for PKC (phospho T514) using **ab109539** at 0.543 µg/ml in Dot blot. Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) was used as the secondary antibody at a dilution of 1/100,000. Exposure time: 180 seconds.

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 (pT500) peptide
Lane 5: Human PKC delta (pT507) peptide
Lane 6: Human PKC eta (pT513) peptide
Lane 7: Human PKC epsilon (pT566) peptide
Lane 8: Human PKC theta (pT538) 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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109539**)



Anti-PKC (phospho T514) antibody [EP2730Y] - BSA and Azide free (ab247898)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors