Product datasheet

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

4 References 10 Images

Overview

Product name Anti-PKC (phospho T514) antibody [EP2730Y]
Description Rabbit monoclonal [EP2730Y] to PKC (phospho T514)
Host species Rabbit
Tested applications Suitable for: WB, IP, IHC-P
Species reactivity Reacts with: Mouse, Rat, Human
Immunogen Synthetic peptide corresponding to Human PKC (phospho T514),
Database link: P05129
Positive control HeLa cell lysate; Human brain tissue; Mouse cerebellum; Rat brain; Human cerebral cortex.
General notes A trial size is available to purchase for this antibody.
Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents.
We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.
This product is a recombinant rabbit monoclonal antibody.

Properties

Form Liquid
Storage buffer pH: 7.20
Preservative: 0.01% Sodium azide
 Constituents: 59% PBS, 40% Glycerol, 0.5% BSA
Purity Protein A purified
Clonality Monoclonal
Clone number EP2730Y
Isotype: IgG

Applications

Our **Abpromise guarantee** covers the use of **ab109539** in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>IP</td>
<td>1/20. For unpurified use at 1/50.</td>
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<tr>
<td>IHC-P</td>
<td>1/300. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. For unpurified use at 1/100 - 1/250.</td>
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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/IκBα). 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**


**Images**

- **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

Blocking and diluting buffer: 5% NFDM/TBST
Immunoprecipitation - Anti-PKC (phospho T514)

antibody [EP2730Y] (ab109539)

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 secondary antibody (HRP) (ab131366) was used as the secondary antibody at 1:1000 dilution. Blocking and diluting buffer: 5% NFDM/TBST.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC (phospho T514)

antibody [EP2730Y] (ab109539)

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 Perform heat mediated antigen retrieval 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 Mouse cerebellum tissue sections labeling PKC with Purified ab109539 at 1:300 dilution (1.14 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval 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 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval 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.
**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:** 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

**Blocking and diluting buffer:** 5% NFDM/TBST

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

**Lane 1:** Active human PKC alpha full length protein.

**Lane 2:** Active human PKC beta 1 full length protein.

**Lane 3:** Active human PKC beta 2 full length protein.

**Lane 4:** Active human PKC delta full length protein.

**Lane 5:** Active human PKC eta full length protein.

**Lane 6:** Active human PKC epsilon full length protein.

**Lane 7:** Active human PKC theta full length protein.

**Lane 8:** Active human PKC mu full length protein.

**Secondary**

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

**Predicted band size:** 78 kDa

**Observed band size:** 100-150 kDa

*why is the actual band size different from the predicted?*

**Exposure time:** 3 minutes
Blocking and Diluting buffer and concentration

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.

Western blot - 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 why is the actual band size different from the predicted?

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.
**Western blot - Anti-PKC (phospho T514) antibody [EP2730Y](ab109539)**

- **All lanes**: Anti-PKC (phospho T514) antibody [EP2730Y](ab109539) at 0.02 µg/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. Why is the actual band size different from the predicted?

**Exposure time**: 15 seconds

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

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

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