# abcam

## Product datasheet

# Anti-PKC gamma (phospho T674) antibody ab5797

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#### Overview

Product name Anti-PKC gamma (phospho T674) antibody

**Description** Rabbit polyclonal to PKC gamma (phospho T674)

Host species Rabbit

**Specificity** PKC alpha (69%) may cross-react in cells expressing a high level of this protein. The peptide

competition data (see Figure) suggest that this antibody will cross-react with PKC alpha [pS657]

(69% homologous), beta 1 [pS661] (54%) and PKC eta [pS674] (38%).

Tested applications Suitable for: WB, IHC-P

Species reactivity Reacts with: Mouse, Human

**Immunogen** Synthetic peptide corresponding to PKC gamma (phospho T674).

**Positive control** WB: HeLa cells treated with PMA, a phorbol ester. IHC-P: Mouse cerebellum tissue, human

cerebellum tissue.

**General notes** 

Protein Kinase C gamma (PKC gamma) is an 80 kDa member of the conventional grou(cPKCs: sensitive to calcium, diacylglycerol, phosphotidylserine and phorbol esters) of the PKC family of serine/threonine kinases that are involved in a wide range of physiological processes including mitogenesis, cell survival and transcriptional regulation. PKC gamma plays a key role in neuronal signal transduction and in regulating intercellular communication. The activation loop threonine (threonine 514 in PKC gamma) of conventional PKCs is phosphorylated by phosphoinositide-dependent kinase-1 (PDK1), which is necessary for its autophosphorylation on threonine 655 in the turn loop, and threonine 674 in the hydrophobic loop of the carboxy terminus, a critical step in generating a catalytically mature enzyme. The phosphorylation of the hydrophobic loop in the carboxyl terminus of PKCs is believed to be a key determinant in regulating PKC interaction with PDK1.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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Form Liquid

**Storage instructions** Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

**Storage buffer** pH: 7.3

Preservative: 0.05% Sodium azide

Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.1% BSA

Purity Immunogen affinity purified

**Purification notes**The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the

site of phosphorylation to remove antibody that is reactive with non-phosphorylated PKC gamma. The final product is generated by affinity chromatography using a PKC gamma-derived peptide

that is phosphorylated at threonine 674.

Primary antibody notes Protein Kinase C gamma (PKC gamma) is an 80 kDa member of the conventional grou(cPKCs:

sensitive to calcium, diacylglycerol, phosphotidylserine and phorbol esters) of the PKC family of serine/threonine kinases that are involved in a wide range of physiological processes including mitogenesis, cell survival and transcriptional regulation. PKC gamma plays a key role in neuronal signal transduction and in regulating intercellular communication. The activation loop threonine (threonine 514 in PKC gamma) of conventional PKCs is phosphorylated by phosphoinositide-dependent kinase-1 (PDK1), which is necessary for its autophosphorylation on threonine 655 in the turn loop, and threonine 674 in the hydrophobic loop of the carboxy terminus, a critical step in generating a catalytically mature enzyme. The phosphorylation of the hydrophobic loop in the carboxyl terminus of PKCs is believed to be a key determinant in regulating PKC interaction with

PDK1.

**Clonality** Polyclonal

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab5797 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	<b>★★★</b> ☆☆ <u>(1)</u>	1/1000. Detects a band of approximately 80 kDa.
IHC-P		1/10 - 1/100.

#### **Target**

**Function** This is a calcium-activated, phospholipid-dependent, serine- and threonine-specific enzyme.

 ${\sf PKC} \ is \ activated \ by \ diacylglycerol \ which \ in \ turn \ phosphorylates \ a \ range \ of \ cellular \ proteins. \ {\sf PKC}$ 

also serves as the receptor for phorbol esters, a class of tumor promoters.

**Tissue specificity** Expressed in Purkinje cells of the cerebellar cortex.

**Involvement in disease** Defects in PRKCG are the cause of spinocerebellar ataxia type 14 (SCA14) [MIM:605361].

Spinocerebellar ataxia is a clinically and genetically heterogeneous group of cerebellar disorders. Patients show progressive incoordination of gait and often poor coordination of hands, speech

and eye movements, due to degeneration of the cerebellum with variable involvement of the

#### Sequence similarities

brainstem and spinal cord. SCA14 is an autosomal dominant cerebellar ataxia (ADCA).

Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. PKC subfamily.

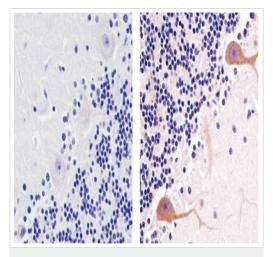
Contains 1 AGC-kinase C-terminal domain.

Contains 1 C2 domain.

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

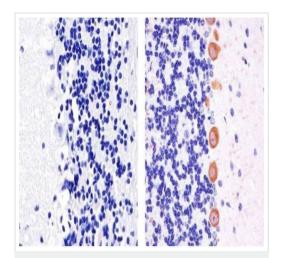
Contains 1 protein kinase domain.

#### **Images**



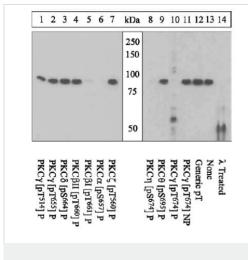
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC gamma (phospho T674) antibody (ab5797)

Paraffin embedded Mouse cerebellum tissue (right) stained for PKC gamma using ab5797 at 1/100 dilution in immunohistochemical analysis. Negative control without primary antibody (left).



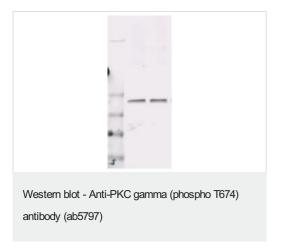
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC gamma (phospho T674) antibody (ab5797)

Paraffin embedded Human cerebellum tissue (right) stained for PKC gamma using ab5797 at 1/20 dilution in immunohistochemical analysis. Negative control without primary antibody (left).



Western blot - Anti-PKC gamma (phospho T674) antibody (ab5797)

Peptide Competition and Phosphatase Treatment: Lysates prepared from HeLa cells stimulated with PMA were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF. Membranes were either left untreated (1-13) or treated with lambda (ë) phosphatase (14), blocked with a 5% BSA-TBST buffer overnight at 4°C, and incubated with 0.50 µg/mL ab5797 antibody for two hours at room temperature in a 3% BSA TBST buffer. following prior incubation with: the phosphopeptide corresponding to the immunogen from other PKC isoforms (1-9), the phosphopeptide immunogen (10), the non-phosphopeptide corresponding to the immunogen (11), a generic phosphothreonine containing peptide (12) or, no peptide (13, 14). After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG HRPconjugate and bands were detected using the Pierce SuperSignalTM method. The data show that the peptide corresponding to PKC gamma [pT674] blocks the antibody signal. The pept



WB from review by Todd O'Buckley submitted 9 April 2004.

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