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#8713

Phospho-PLC γ 1 (Ser1248) (D25A9) Rabbit mAb

For Research Use Only. Not for Use in Diagnostic Procedures.

Applications: W, W-S, IP, IHC-P, IF-IC, FC-FP	Reactivity: H M Mk	Sensitivity: Endogenous	MW (kDa): 150	Source/Isotype: Rabbit IgG	UniProt ID: #P19174	Entrez-Gene Id: 5335
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Product Usage Information

Application

Western Blotting
Simple Western™
Immunoprecipitation
Immunohistochemistry (Paraffin)
Immunofluorescence (Immunocytochemistry)
Flow Cytometry (Fixed/Permeabilized)

Dilution

1:1000
1:10 - 1:50
1:50
1:100 - 1:400
1:100 - 1:200
1:800

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

Specificity/Sensitivity

Phospho-PLC γ 1 (Ser1248) (D25A9) Rabbit mAb recognizes endogenous levels of PLC γ 1 protein only when phosphorylated at Ser1248.

Species predicted to react based on 100% sequence homology

Rat

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser1248 of human PLC γ 1 protein.

Background

Phosphoinositide-specific phospholipase C (PLC) plays a significant role in transmembrane signaling. In response to extracellular stimuli, such as hormones, growth factors, and neurotransmitters, PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP₂) to generate two secondary messengers: inositol 1,4,5-triphosphate (IP₃) and diacylglycerol (DAG) (1). At least four families of PLCs have been identified: PLC β , PLC γ , PLC δ , and PLC ϵ . Phosphorylation is one of the key mechanisms that regulate the activity of PLC. PLC γ is activated by both receptor and non-receptor tyrosine kinases (2). PLC γ forms a complex with EGF and PDGF receptors, which leads to the phosphorylation of PLC γ at Tyr771, 783, and 1248 (3). Phosphorylation by Syk at Tyr783 activates the enzymatic activity of PLC γ 1 (4). PLC γ 2 is engaged in antigen-dependent signaling in B cells and collagen-dependent signaling in platelets. Phosphorylation by Btk or Lck at Tyr753, 759, 1197, and 1217 is correlated with PLC γ 2 activity (5,6).

Two mammalian PLC γ isoforms (γ 1 and γ 2) have been cloned and characterized (7,8). Like other PLC-family members, PLC γ 1 and PLC γ 2 contain calcium-binding (EF-hand, C2) and lipid-interacting (PH, EF-hand) domains necessary for their enzymatic activity and substrate recognition. Uniquely, PLC γ isoforms have additional, conserved SH2 and SH3 domains critical for their functions as signaling molecules and scaffolding proteins. Upon growth factor stimulation, PLC γ 1 is recruited (via SH2 domains) to phosphotyrosine residues within the cytoplasmic tail of many RTKs where it serves as a substrate for the RTK and provides docking sites for additional proteins involved in RTK signaling (4-6,9-12). PLC γ 1 and γ 2 can also be activated downstream of receptors lacking intrinsic tyrosine kinase activity. This has been reported downstream of multiple G protein-coupled receptors and the T cell receptor in which tyrosine kinases of the Src, Syk, and Tec families serve to bind, phosphorylate, and activate PLC γ (reviewed in 13-15). Phosphorylation at tyrosine residues by both receptor and non-receptor tyrosine kinases results in robust activation of PLC γ 1 activity, leading to generation of second messengers. In response to agonists, PLC γ 1 is phosphorylated on Tyr783, Tyr711, and Tyr1253 (Tyr753, Tyr759, and Tyr1217 in PLC γ 2) resulting in robust PI-4,5-P₂ hydrolysis (4-6,9-12). Interestingly recent evidence suggests a role for tyrosine kinase-independent regulation of PLC γ in some systems. For example, in response to EGF, proline-rich regions of Akt interact with the SH3 domain of PLC γ 1 resulting in association of the two enzymes, phosphorylation of PLC γ 1 at Ser1248, and enhanced cellular motility (16). This finding demonstrates that PLC γ 1 can function as a "scaffold" between RTKs and Akt, thereby establishing a mechanism by which the Akt signaling pathway cross-talks with tyrosine kinases. However, the mechanism and functional significance of phosphorylation at Ser1248 remains to be fully clarified, as it has also been shown that PKA-mediated phosphorylation at this site is

inhibitory to PLC γ 1 tyrosine phosphorylation and phospholipase activity in CD3-treated Jurkat cells (17), suggesting that Ser1248 may be an allosteric regulator of PLC γ 1 activity.

Background References

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Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween@ 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **W-S:** Simple Western™ **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin) **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: Human **M:** Mouse **Mk:** Monkey

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