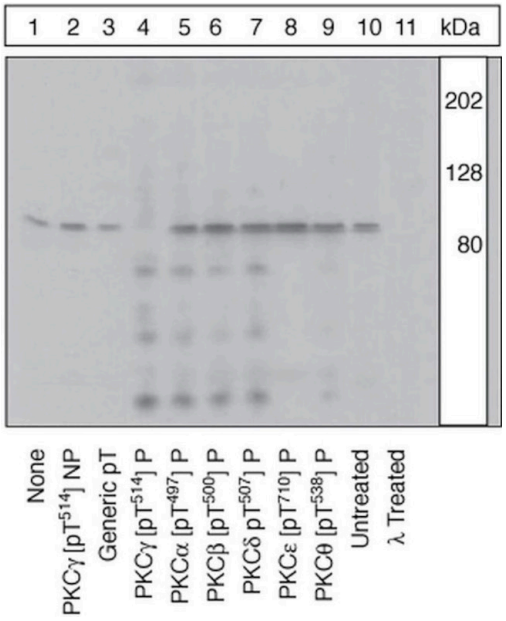
Phospho-PKC gamma (Thr514) Polyclonal Antibody

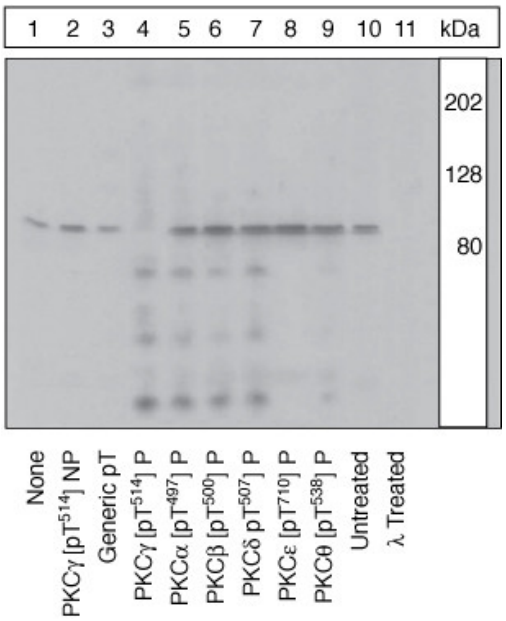
Product Details	
Size	100 µL
Species Reactivity	Human, Mouse
Published Species	Hamster
Host/Isotype	Rabbit / IgG
Class	Polyclonal
Type	Antibody
Conjugate	Unconjugated
Immunogen	The antiserum was produced against a chemically synthesized phosphopeptide derived from a region of human PKC-gamma that contains threonine 514
Form	Liquid
Purification	Antigen affinity chromatography
Storage buffer	Dulbecco's PBS, pH 7.3, with 1mg/mL BSA
Contains	0.05% sodium azide
Storage conditions	-20°C
RRID	AB_2533806

Applications	Tested Dilution	Publications
Western Blot (WB)	1:1,000	2 Publications
Immunohistochemistry (Paraffin) (IHC (P))	1:10-1:100	-

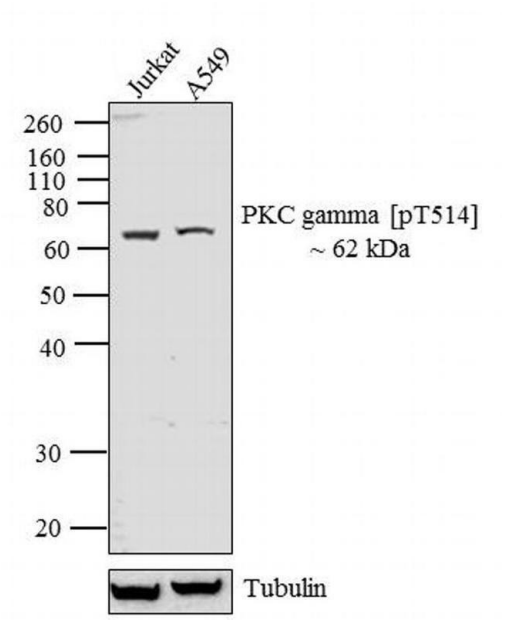
Product Images For Phospho-PKC gamma (Thr514) Polyclonal Antibody



Phospho-PKC gamma (Thr514) Antibody (44-956)
Antibody specificity is demonstrated by Peptide Array. Western blotting of Phospho-PKC gamma (Thr514) using anti-Phospho-PKC gamma (Thr514) Polyclonal Antibody (Product # 44-956) shows loss of signal with the specific peptide and not with other relevant peptides. {ARRAY}



Phospho-PKC gamma (Thr514) Antibody (44-956) in WB
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 (1-10) or treated with lambda phosphatase (11), blocked with a 3% low-fat milk-TBST buffer overnight at 4°C, and incubated with 0.50 µg/mL PKCgamma (pT514) antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 10, 11), the non-phosphopeptide corresponding to the immunogen (2), a generic phosphothreonine containing peptide (3), the phosphopeptide immunogen (4), or, the phosphopeptide corresponding to the immunogen from other PKC isoforms (5-9). After washing, membranes were incubated with goat F (ab')2 anti-rabbit IgG alkaline phosphatase (Product # ALI4405) and signals were detected using the Tropix WesternStar™ method. The data show that the peptide corresponding to PKCgamma (pS514) blocks the antibody signal and that the peptides corresponding to PKC isoforms alpha (pT497), betaI&II (pT500), delta (pT507), epsilon (pT710) and theta (pT538) did not block the antibody signal, thereby demonstrating the specificity of the antibody. The data also show that phosphatase stripping eliminates the signal, verifying that the antibody is phospho-specific.



Phospho-PKC gamma (Thr514) Antibody (44-956) in WB
Western blot analysis was performed on whole cell extracts (30 µg lysate) of Jurkat (Lane 1) and A549 (Lane 2). The blots were probed with Anti-Phospho-PKC-gamma (Thr514) Rabbit Polyclonal Antibody (Product # 44-956, 1:500 dilution) and detected by chemiluminescence using Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Secondary Antibody, HRP conjugate (Product # A27036, 0.4 µg/mL, 1:2500 dilution). A 62 kDa band corresponding to PKC-gamma (pT514) was observed across treated cell lines tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 12 % Bis-Tris gel (Product # NP0342BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).

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2 References

Western Blot (2)

The Journal of biological chemistry

Mutant protein kinase Cgamma found in spinocerebellar ataxia type 14 is susceptible to aggregation and causes cell death.

"44-956 was used in Western Blotting to indicate that spinocerebellar ataxia type 14 (SCA14) mutations make protein kinase Cgamma (gammaPKC) form cytoplasmic aggregates, suggesting the involvement of this property in the etiology of SCA14."

Authors: Seki T,Adachi N,Ono Y,Mochizuki H,Hiramoto K,Amano T,Matsubayashi H,Matsumoto M,Kawakami H,Saito N, Sakai N

Year
2005

Species
Hamster

Dilution
1:1000

Genes to cells : devoted to molecular & cellular mechanisms

Phosphorylation of PKC activation loop plays an important role in receptor-mediated translocation of PKC.

Authors: Seki T,Matsubayashi H,Amano T,Shirai Y,Saito N,Sakai N

Year
2005

Species
Hamster