# Anti-Phospho-PKC alpha (T638) Antibody [JF0964] ET1702-17

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|---|---|
| **Product Type:** | Recombinant Rabbit monoclonal IgG, primary antibodies |
| **Species reactivity:** | Human, Mouse, Rat |
| **Applications:** | WB, IF-Cell, IF-Tissue, IHC-P, IP, Dot Blot |
| **Molecular Wt:** | Predicted band size: 77 kDa |
| **Clone number:** | JF0964 |

**Description:** Members of the protein kinase C (PKC) family play a key regulatory role in a variety of cellular functions including cell growth and differentiation, gene expression, hormone secretion and membrane function. PKCs were originally identified as serine/threonine protein kinases whose activity was dependent on calcium and phospholipids. Diacylglycerols (DAG) and tumor-promoting phorbol esters bind to and activate PKC. PKCs can be subdivided into many different isoforms (α, βI, βII, γ, δ, ε, ζ, η, θ, λ/ι, μ and ν). Patterns of expression for each PKC isoform differ among tissues and PKC family members exhibit clear differences in their cofactor dependencies. For instance, the kinase activities of PKC δ and ε are independent of $Ca^{2+}$. On the other hand, most of the other PKC members possess phorbol ester-binding activities and kinase activities.

**Immunogen:** Synthetic phospho-peptide corresponding to residues surrounding Thr638 of Human PKC alpha aa 622-667 / 672.

**Positive control:** HEK-293 cell lysate, HeLa cell lysate, NIH/3T3 cell lysate, C6 cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, NIH/3T3 starved overnight then treated with 200nM TPA for 4 hours cell lysate, HeLa, NIH/3T3, C6, human breast cancer tissue, mouse brain tissue, rat brain tissue.

**Subcellular location:** Cytoplasm, Cell membrane, Mitochondrion membrane, Nucleus.

**Database links:** SwissProt: P17252 Human | P20444 Mouse | P05696 Rat

**Recommended Dilutions:**

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| **WB** | 1:5,000 |
| **IF-Cell** | 1:50-1:500 |
| **IF-Tissue** | 1:50-1:500 |
| **IHC-P** | 1:50-1:1,000 |
| **IP** | Use at an assay dependent concentration. |
| **Dot Blot** | 1:5,000 |

**Storage Buffer:** 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

**Storage Instruction:** Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

**Purity:** Protein A affinity purified.

## Images

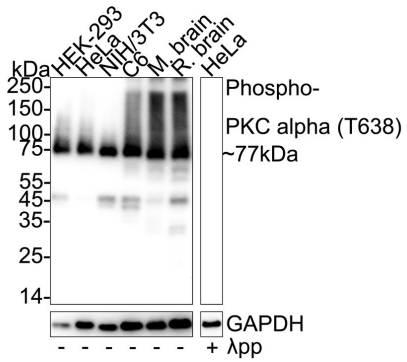


**Fig1:** Western blot analysis of Phospho-PKC alpha (T638) on different lysates with Rabbit anti-Phospho-PKC alpha (T638) antibody (ET1702-17) at 1/5,000 dilution.

Lane 1: HEK-293 cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)
Lane 3: NIH/3T3 cell lysate (20 µg/Lane)
Lane 4: C6 cell lysate (20 µg/Lane)
Lane 5: Mouse brain tissue lysate(40 µg/Lane)
Lane 6: Rat brain tissue lysate(40 µg/Lane)
Lane 3: HeLa cell lysate, the membrane treated with λpp for 1 hour (20 µg/Lane)

Predicted band size: 77 kDa
Observed band size: 77 kDa

Exposure time: 10 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1702-17) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

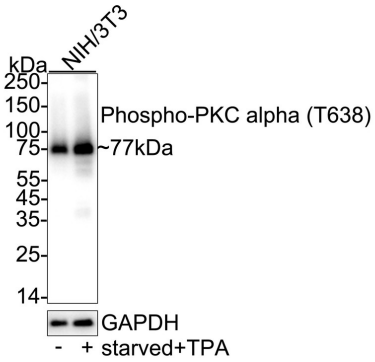


**Fig2:** Western blot analysis of Phospho-PKC alpha (T638) on different lysates with Rabbit anti-Phospho-PKC alpha (T638) antibody (ET1702-17) at 1/5,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: NIH/3T3 starved overnight then treated with 200nM TPA for 4 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 77 kDa
Observed band size: 77 kDa

Exposure time: 10 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1702-17) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

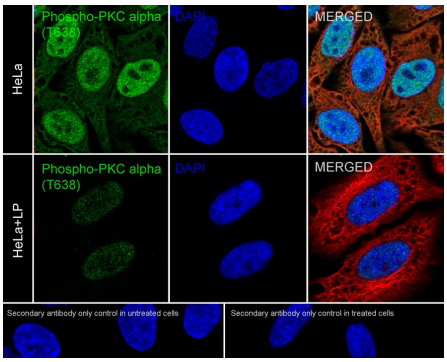


**Fig3:** Immunocytochemistry analysis of HeLa cells treated with or without λpp labeling Phospho-PKC alpha (T638) with Rabbit anti-Phospho-PKC alpha (T638) antibody (ET1702-17) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-PKC alpha (T638) antibody (ET1702-17) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

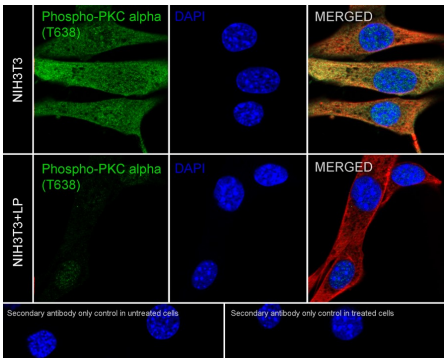


**Fig4:** Immunocytochemistry analysis of NIH/3T3 cells treated with or without λpp labeling Phospho-PKC alpha (T638) with Rabbit anti-Phospho-PKC alpha (T638) antibody (ET1702-17) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-PKC alpha (T638) antibody (ET1702-17) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

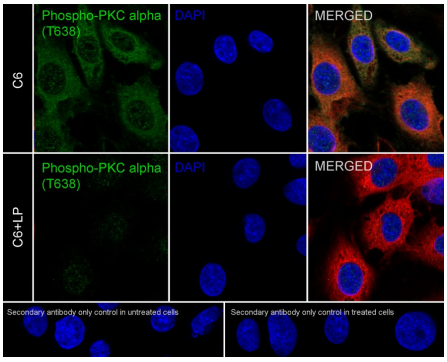


**Fig5:** Immunocytochemistry analysis of C6 cells treated with or without λpp labeling Phospho-PKC alpha (T638) with Rabbit anti-Phospho-PKC alpha (T638) antibody (ET1702-17) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-PKC alpha (T638) antibody (ET1702-17) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

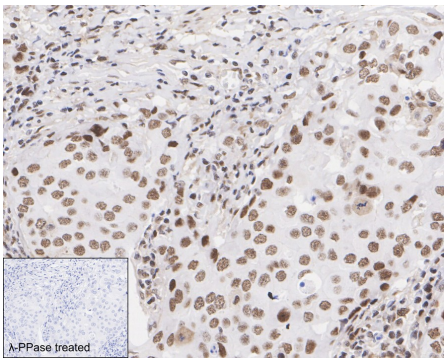


**Fig6:** Immunohistochemical analysis of paraffin-embedded human breast cancer tissue untreated / treated with λpp with Rabbit anti-Phospho-PKC alpha (T638) antibody (ET1702-17) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with $ddH_2O$ and PBS, and then probed with the primary antibody (ET1702-17) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

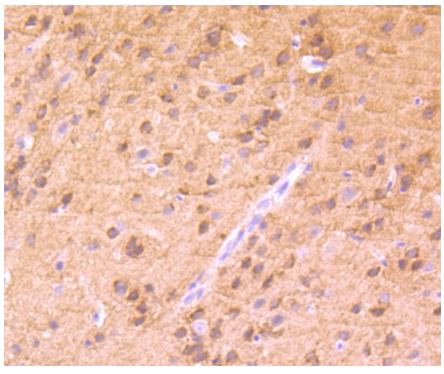

**Fig7:** Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-Phospho-PKC alpha (T638) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with $ddH_2O$ and PBS, and then probed with the primary antibody (ET1702-17, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

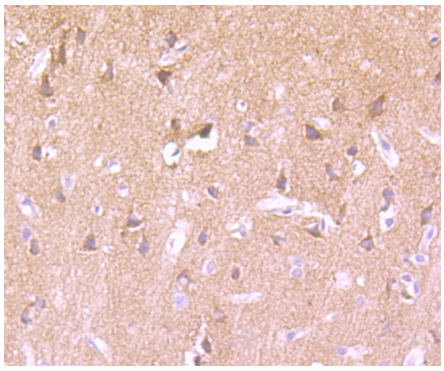

**Fig8:** Immunohistochemical analysis of paraffin-embedded rat brain tissue using anti-Phospho-PKC alpha (T638) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (ET1702-17, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

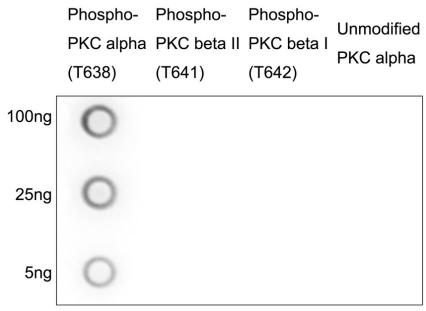


**Fig9:** Dot blot analysis of Phospho-PKC alpha (T638) on different peptides with Rabbit anti-Phospho-PKC alpha (T638) antibody (ET1702-17) at 1/5,000 dilution. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution for 1 hour at room temperature.

Lane 1: Phospho-PKC alpha (T638) peptide (positive)
Lane 2: Phospho-PKC beta II (T641) peptide (negative)
Lane 3: Phospho-PKC beta I (T642) peptide (negative)
Lane 4: Unmodified PKC alpha peptide (negative)

Proteins loading: 100ng, 25ng, 5ng;

Blocking and dilution buffer: 5% NFDM/TBST;

Exposure time: 3 seconds; ECL: K1801.



## Background References

1. Wang XH et al. Cannabinoid CB1 receptor signaling dichotomously modulates inhibitory and excitatory synaptic transmission in rat inner retina. Brain Struct Funct 221:301-16 (2016).
2. Griffon A et al. Integrative analysis of public ChIP-seq experiments reveals a complex multi-cell regulatory landscape. Nucleic Acids Res 43:e27 (2015).