Anti-PKC alpha Antibody [SU31-08]

ET1608-15

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 77 kDa
Clone number:	SU31-08

Description: Protein kinase C alpha (PKCα) is an enzyme that in humans is encoded by the PRKCA gene. Protein kinase C (PKC) is a family of serine- and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol. PKC family members phosphorylate a wide variety of protein targets and are known to be involved in diverse cellular signaling pathways. PKC family members also serve as major receptors for phorbol esters, a class of tumor promoters. Each member of the PKC family has a specific expression profile and is believed to play a distinct role in cells. The protein encoded by this gene is one of the PKC family members. This kinase has been reported to play roles in many different cellular processes, such as cell adhesion, cell transformation, cell cycle checkpoint, and cell volume control. Knockout studies in mice suggest that this kinase may be a fundamental regulator of cardiac contractility and Ca2+ handling in myocytes. Protein kinase C-alpha (PKC-α) is a specific member of the protein kinase family. These enzymes are characterized by their ability to add a phosphate group to other proteins, thus changing their function.

Immunogen: Recombinant protein within Human PKC alpha aa 560-672 / 672.

Positive control: 293T cell lysate, U-87 MG cell lysate, NIH/3T3 cell lysate, C6 cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, Hela, MCF-7, CRC, A549, PC-12, 293T, NIH/3T3, C6.

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

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

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
FC	1:50-1:100
IP	1:10-1:50

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

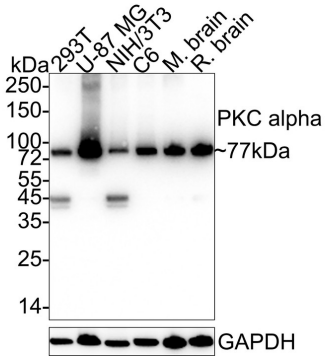


Fig1: Western blot analysis of PKC alpha on different lysates with Rabbit anti-PKC alpha antibody (ET1608-15) at 1/1,000 dilution.

Lane 1: 293T cell lysate (20 µg/Lane)
Lane 2: U-87 MG 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)

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

Exposure time: 9 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 (ET1608-15) at 1/1,000 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.

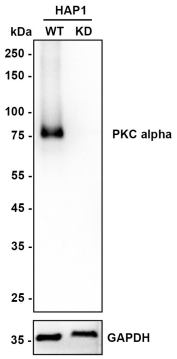


Fig2: Western blot analysis of PKC alpha on different lysates with Rabbit anti-PKC alpha antibody (ET1608-15) at 1/2,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-PKC alpha KD cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 40 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 (ET1608-15) at 1/2,000 dilution was used in 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.

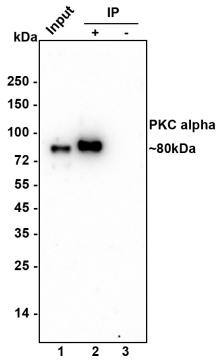
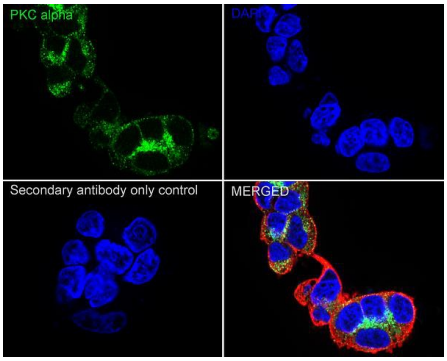


Fig3: PKC alpha was immunoprecipitated from 0.2 mg 293T cell lysate with ET1608-15 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1608-15 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: 293T cell lysate (input)
Lane 2: ET1608-15 IP in 293T cell lysate
Lane 3: Rabbit IgG instead of ET1608-15 in 293T cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 2 min; ECL: K1801

Fig4: Immunocytochemistry analysis of 293T cells labeling PKC alpha with Rabbit anti-PKC alpha antibody (ET1608-15) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-PKC alpha antibody (ET1608-15) 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 (HA601187, 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.

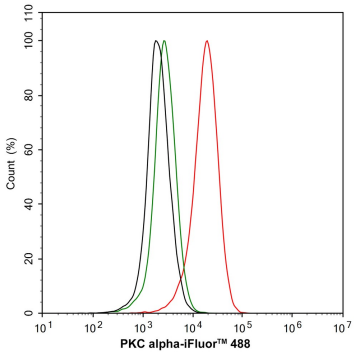


Fig5: Flow cytometric analysis of 293T cells labeling PKC alpha.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1608-15, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

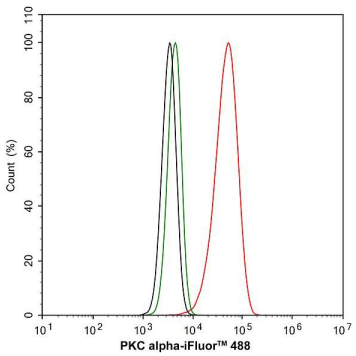


Fig6: Flow cytometric analysis of NIH/3T3 cells labeling PKC alpha.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1608-15, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

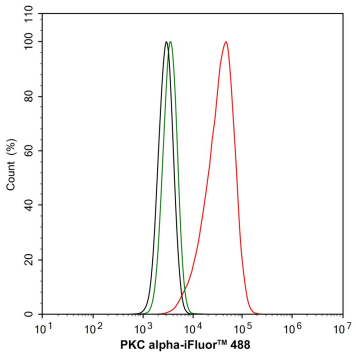


Fig7: Flow cytometric analysis of C6 cells labeling PKC alpha.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1608-15, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

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. Cao Y et al. Regulators of G protein signaling RGS7 and RGS11 determine the onset of the light response in ON bipolar neurons. Proc Natl Acad Sci U S A 109:7905-10 (2012).