

## Anti-Phospho-PKC alpha/beta II (T638/T641) Antibody [PSH15-91]

# HA723796



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	WB, IF-Cell, Dot Blot
<b>Molecular Wt:</b>	Predicted band size: 77 kDa
<b>Clone number:</b>	PSH15-91

**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 ( $\alpha$ ,  $\beta$ I,  $\beta$ II,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$ ,  $\eta$ ,  $\theta$ ,  $\lambda$ /I,  $\mu$  and  $\nu$ ). 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  $\delta$  and  $\epsilon$  are independent of  $\text{Ca}^{2+}$ . 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, C6 cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, NIH/3T3 cell lysate, NIH/3T3 starved overnight then treated with 200nM TPA for 4 hours cell lysate, HeLa, NIH/3T3, C6.

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

**Database links:** SwissProt: P17252 Human | P05771-2 Human | P20444 Mouse | P68404-2 Mouse | P05696 Rat | P68403-2 Rat

### Recommended Dilutions:

<b>WB</b>	1:250,000
<b>IF-Cell</b>	1:100
<b>Dot Blot</b>	1:5,000

**Storage Buffer:** 1\*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

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

**Purity:** Protein A affinity purified.

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Orders:0086-571-88062880

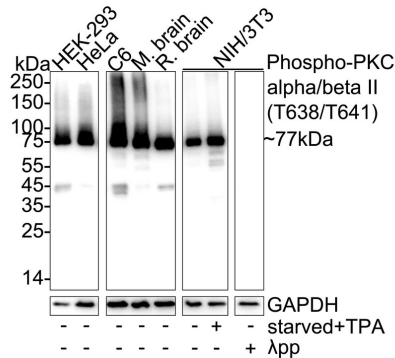
Technical:0086-571-89986345

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Images

**Fig1:** Western blot analysis of Phospho-PKC alpha/beta II (T638/T641) on different lysates with Rabbit anti-Phospho-PKC alpha/beta II (T638/T641) antibody (HA723796) at 1/25,000 dilution.



Lane 1: HEK-293 cell lysate

Lane 2: HeLa cell lysate

Lane 3: C6 cell lysate

Lane 4: Mouse brain tissue lysate

Lane 5: Rat brain tissue lysate

Lane 6: NIH/3T3 cell lysate

Lane 7: NIH/3T3 starved overnight then treated with 200nM TPA for 4 hours cell lysate

Lane 8: NIH/3T3 starved overnight then treated with 200nM TPA for 4 hours cell lysate, then the membrane treated with  $\lambda$ pp for 1 hour

Lysates/proteins at 20  $\mu$ g/Lane.

Predicted band size: 77 kDa

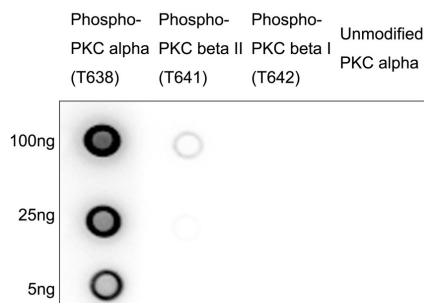
Observed band size: 77 kDa

Exposure time: 14 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 (HA723796) at 1/25,000 dilution was used in primary antibody dilution (K1803) at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

**Fig2:** Dot blot analysis of Phospho-PKC alpha/beta II (T638/T641) on different peptides with Rabbit anti-Phospho-PKC alpha/beta II (T638/T641) antibody (HA723796) 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 (positive)

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.

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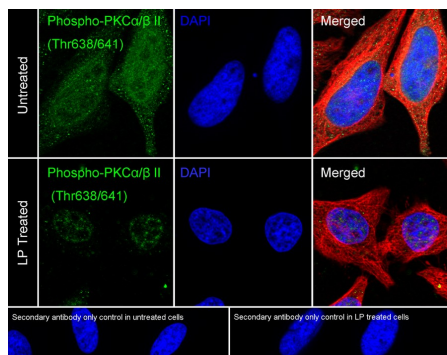
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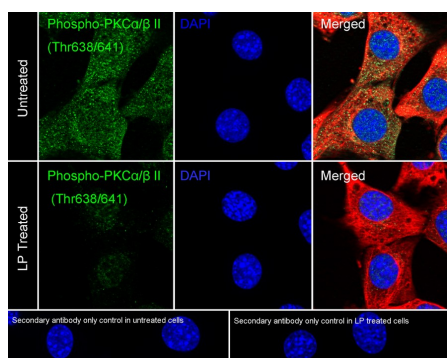
**Fig3:** Immunocytochemistry analysis of HeLa cells untreated / treated with  $\lambda$ pp labeling Phospho-PKC alpha/beta II (T638/T641) with Rabbit anti-Phospho-PKC alpha/beta II (T638/T641) antibody (HA723796) at 1/100 dilution.



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-Phospho-PKC alpha/beta II (T638/T641) antibody (HA723796) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

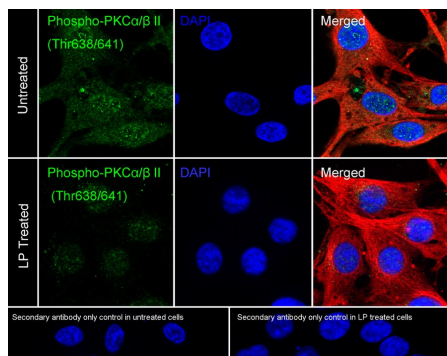
**Fig4:** Immunocytochemistry analysis of NIH/3T3 cells untreated / treated with  $\lambda$ pp labeling Phospho-PKC alpha/beta II (T638/T641) with Rabbit anti-Phospho-PKC alpha/beta II (T638/T641) antibody (HA723796) at 1/100 dilution.



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-Phospho-PKC alpha/beta II (T638/T641) antibody (HA723796) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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 °C. 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 untreated / treated with  $\lambda$ pp labeling Phospho-PKC alpha/beta II (T638/T641) with Rabbit anti-Phospho-PKC alpha/beta II (T638/T641) antibody (HA723796) 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-Phospho-PKC alpha/beta II (T638/T641) antibody (HA723796) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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

## Background References

1. Ghashghaeinia M et al. Coronavirus disease 2019 (COVID-19), human erythrocytes and the PKC-alpha/-beta inhibitor chelerythrine -possible therapeutic implication. Cell Cycle. 2020 Dec

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