# Anti-Phospho-AKT2 (S474) Antibody [PSH09-46] HA723108

| Product Type:                      | Recombinant Rabbit monoclonal IgG, primary antibodies   |
|------------------------------------|---|
| Species reactivity:                | Human, Mouse, Rat   |
| Applications:                      | WB, FC  |
| Molecular Wt:                      | Predicted band size: 56 kDa   |
| Clone number:                      | PSH09-46  |
| Description:                       | AKT2, also known as RAC-beta serine/threonine-protein kinase, is an enzyme that in humans is encoded by the AKT2 gene. It influences metabolite storage as part of the insulin signal transduction pathway. This gene is a putative oncogene encoding a protein belonging to the AKT subfamily of serine/threonine kinases that contain SH2-like (Src homology 2-like) domains. The encoded protein is a general protein kinase capable of phosphorylating several known proteins. AKT2 has important roles in controlling glycogenesis, gluconeogenesis, and glucose transport as part of the insulin signal transduction pathway. |
| lmmunogen:                         | Synthetic phospho-peptide corresponding to residues surrounding Ser474 of human AKT2.   |
| Positive control:                  | MCF7 cell lysate, MCF7 treated with 50ng/mL Calyculin A for 45 minutes cell lysate, HEK-293 cell lysate, HEK-293 treated with 50µM LY294002 for 6 hours cell lysate, NIH/3T3 treated with 100ng/mL PDGF for 1 hour cell lysate, C6 cell lysate, C6 treated with 100ng/mL Calyculin A for 1 hour cell lysate, MCF7 cells treated with 50ng/mL Calyculin A for 45 minutes, C6 cells treated with 100ng/mL Calyculin A for 1 hours.  |
| Subcellular location:              | Cytoplasm, Nucleus, Cell membrane, Early endosome.  |
| Database links:                    | SwissProt: P31751 Human   Q60823 Mouse   P47197 Rat   |
| Recommended Dilutions:<br>WB<br>FC | 1:2,000<br>1:1,000  |
| Storage Buffer:                    | PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.  |
| Storage Instruction:               | Store at +4 $^\circ\!\!\mathbb{C}$ after thawing. Aliquot store at -20 $^\circ\!\!\mathbb{C}$ . Avoid repeated freeze / thaw cycles.  |
| Purity:                            | Protein A affinity purified.  |
|                                    |   |

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Orders:0086-571-88062880

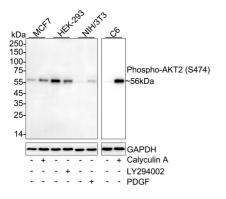
Technical:0086-571-89986345

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

#### Images



**Fig1:** Western blot analysis of Phospho-AKT2 (S474) on different lysates with Rabbit anti-Phospho-AKT2 (S474) antibody (HA723108) at 1/2,000 dilution.

Lane 1: MCF7 cell lysate

Lane 2: MCF7 treated with 50ng/mL Calyculin A for 45 minutes cell lysate

Lane 3: HEK-293 cell lysate

Lane 4: HEK-293 treated with 50 $\mu\text{M}$  LY294002 for 6 hours cell lysate

Lane 5: NIH/3T3 cell lysate

Lane 6: NIH/3T3 treated with 100ng/mL PDGF for 1 hour cell lysate

Lane 7: C6 cell lysate

Lane 8: C6 treated with 100ng/mL Calyculin A for 1 hour cell lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 56 kDa Observed band size: 56 kDa

Exposure time: Lane 1-6: 3 minutes; Lane 7-8: 12 seconds; ECL: K1802;

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 (HA723108) at 1/2,000 dilution was used in 5% NFDM/TBST at  $4^{\circ}$ C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

**Fig2:** Flow cytometric analysis of MCF7 cells (left) / MCF7 cells treated with 50ng/mL Calyculin A for 45 minutes (right) labeling Phospho-AKT2 (S474).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723108, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor TM 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 °C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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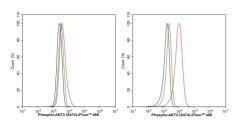
10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup> 1 ho-AKT2 (S474)-IFluor<sup>™</sup> 488

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**Fig3:** Flow cytometric analysis of C6 cells (left) / C6 cells treated with 100ng/mL Calyculin A for 1 hours (right) labeling Phospho-AKT2 (S474).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723108, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor TM 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 °C. 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. Feng SY et al. Increased joint loading induces subchondral bone loss of the temporomandibular joint via the RANTES-CCRs-Akt2 axis. JCI Insight. 2022 Nov
- Ghosh S et al. The AKT2/SIRT5/TFEB pathway as a potential therapeutic target in atrophic AMD. bioRxiv [Preprint].
  2023 Aug

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