Product Datasheet

Biological Description

Specificity	Phospho-Akt1 (Ser129) Antibody [K23C4] detects endogenous levels of total Akt1 protein only when it is phosphorylated at Ser129.
Background	Phospho-Akt1 (Ser129) is a specific post-translational modification of Akt1 (RAC-alpha serine/threonine-protein kinase, PKBα), occurring within the flexible linker region between the pleckstrin homology (PH) domain and the kinase domain of this 480-amino-acid protein. Akt1 comprises an N-terminal PH domain for PIP3-mediated membrane recruitment, a catalytic kinase domain with key regulatory phosphorylation sites at Thr308 (activation loop) and Ser473 (hydrophobic motif), and a C-terminal regulatory tail. Phosphorylation of Ser129 by protein kinase CK2 generates a consensus S-x-x-D/E motif that primes subsequent modification at Ser126 and stabilizes the overall conformation of Akt1. This modification hyperactivates Akt1 beyond the effects of canonical PDK1/mTORC2 phosphorylation by strengthening its association with the HSP90 chaperone complex, which protects Thr308 from PP2A-mediated dephosphorylation, thereby sustaining Akt1 kinase activity. Additionally, phospho-Ser129 enhances β-catenin/TCF transcriptional activity, either directly or by stabilizing Wnt signaling components, and locks Akt1 in an extended active conformation that allows for isoform-specific substrate selection, such as preferential phosphorylation of palladin, a process not mirrored in Akt2 due to the absence of the equivalent Ser131 site. This modification drives cytoskeletal remodeling, cell survival through inhibition of Bad and FoxO, proliferation by promoting cyclin D1 stability and p27/p21 sequestration, and mTORC1 activation via TSC2 phosphorylation, with hierarchical phosphorylation ensuring robust signal amplification upon growth factor stimulation. In disease, elevated phospho-Ser129 levels enhance cancer cell survival and metastasis by amplifying PI3K/Akt oncogenic signaling (notably in breast and prostate cancers), contribute to chemoresistance via sustained HSP90 protection, and are implicated in metabolic disorders through disruption of glucose homeostasis by hyperactivating Akt1.

Usage Information

Application	WB	Dilution	WB
Reactivity	Human		1:1000 - 1:10000
Source	Rabbit Monoclonal Antibody	MW	55 kDa
Storage Buffer	PBS, pH 7.2+50% Glycerol+0.05% BSA+0.01% NaN3		
Storage (from the date of receipt)	-20°C (avoid freeze-thaw cycles), 2 years		

Experimental Methods

WB

Experimental Protocol:

Sample preparation
1. Tissue: Lyse the tissue sample by adding an appropriate volume of ice-cold **RIPA/NP-40 Lysis Buffer (containing Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail)**, and homogenize the tissue at a low temperature or lyse it by sonication on ice, then incubate on ice for 30 minutes.
2. Adherent cell: Aspirate the culture medium and wash the cells with ice-cold PBS twice. Lyse the cells by adding an appropriate volume of **RIPA/NP-40 Lysis Buffer (containing Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail)**, sonicate to lyse the cells, and incubate on ice for 30 minutes.
3. Suspension cell: Transfer the culture medium to a pre-cooled centrifuge tube. Centrifuge and aspirate the supernatant. Wash the cells with ice-cold PBS twice. Lyse the cells by adding an appropriate volume of **RIPA/NP-40 Lysis Buffer (containing Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail)**, sonicate to lyse the cells, and incubate on ice for 30 minutes.
4. Place the lysate into a pre-cooled microcentrifuge tube. Centrifuge at 4°C for 15 min. Collect the supernatant;
5. Remove a small volume of lysate to determine the protein concentration;
6. Combine the lysate with protein loading buffer. Boil 20 μL sample under 95-100°C for 5 min. Centrifuge for 5 min after cool down on ice.

Electrophoretic separation
1. According to the concentration of extracted protein, load appropriate amount of protein sample and marker onto SDS-PAGE gels for electrophoresis. Recommended separating gel (lower gel) concentration: 10%. Reference Table for Selecting SDS-PAGE Separation Gel Concentrations
2. Power up 80V for 30 minutes. Then the power supply is adjusted (110 V~150 V), the Marker is observed, and the electrophoresis can be stopped when the indicator band of the predyed protein Marker where the protein is located is properly separated. (Note that the current should not be too large when electrophoresis, too large current (more than 150 mA) will cause the temperature to rise, affecting the result of running glue. If high currents cannot be avoided, an ice bath can be used to cool the bath.)

Transfer membrane
1. Take out the converter, soak the clip and consumables in the pre-cooled converter;
2. Activate PVDF membrane with methanol for 1 min and rinse with transfer buffer;
3. Install it in the order of "black edge of clip - sponge - filter paper - filter paper - glue -PVDF membrane - filter paper - filter paper - sponge - white edge of clip";
4. The protein was electrotransferred to PVDF membrane. (0.45 μm PVDF membrane is recommended) Reference Table for Selecting PVDF Membrane Pore Size Specifications
Recommended conditions for wet transfer: **200 mA, 120 min**.
(Note that the transfer conditions can be adjusted according to the protein size. For high-molecular-weight proteins, a higher current and longer transfer time are recommended. However, ensure that the transfer tank remains at a low temperature to prevent gel melting.)

Block
1. After electrotransfer, wash the film with TBST at room temperature for 5 minutes;
2. Incubate the film in the blocking solution **(recommending 5% BSA solution)** for 1 hour at room temperature;
3. Wash the film with TBST for 3 times, 5 minutes each time.

Antibody incubation
1. Use 5% skim milk powder to prepare the primary antibody working liquid (recommended dilution ratio for primary antibody 1:1000), gently shake and incubate with the film at 4°C overnight;
2. Wash the film with TBST 3 times, 5 minutes each time;
3. Add the secondary antibody to the blocking solution and incubate with the film gently at room temperature for 1 hour;
4. After incubation, wash the film with TBST 3 times for 5 minutes each time.

Antibody staining
1. Add the prepared ECL luminescent substrate (or select other color developing substrate according to the second antibody) and mix evenly;
2. Incubate with the film for 1 minute, remove excess substrate (keep the film moist), wrap with plastic film, and expose in the imaging system.

References

https://pubmed.ncbi.nlm.nih.gov/24769357/ https://pubmed.ncbi.nlm.nih.gov/15818404/

Application Data

WB

Validated by Capsid Bio

Lane 1: MCF-7, Lane 2: MCF-7 (Alkaline Phosphatase treated)