Product Datasheet

Biological Description

Specificity	Phospho-p130 (Ser672) Antibody [E21C6] detects endogenous levels of total p130 protein only when it is phosphorylated at Ser672.
Background	Phospho-p130 (Ser672) refers to the retinoblastoma-related protein p130 (RBL2), a crucial tumor suppressor of the pocket protein family (including pRb and p107) that enforces G0/G1 cell cycle arrest by binding and repressing E2F transcription factors. p130 contains conserved N-terminal, spacer, and C-terminal regions, featuring a large A/B pocket domain (the cyclin-binding motif) and multiple phosphorylation sites targeted by cyclin-dependent kinases (CDKs). Among these, Ser672 in the C-terminus is a CDK4/6-specific phosphorylation site pivotal for proteasomal degradation. During the late G1/S phase, phosphorylation at Ser672 by CDK4/6 marks p130 for recognition by the F-box protein Skp2, a component of the SCF^Skp2 ubiquitin ligase complex. This enables p130 polyubiquitination and rapid proteasomal turnover, independently of CDK2 activity. As a result, p130-mediated repression of E2F-responsive genes is relieved, allowing S-phase entry, DNA replication, and cell proliferation, while terminating the growth-suppressive function of p130. In quiescent G0 cells, hypophosphorylated p130 forms stable complexes with E2F4/5-DP1 to silence proliferation-associated genes; phosphorylation at Ser672 dynamically lowers p130 levels as cells re-enter the cycle. Dysregulation of the CDK4/6-Skp2-p130 axis or p130 mutations can stabilize hyperphosphorylated forms and disrupt cell cycle checkpoints, promoting uncontrolled proliferation and cancer development.

Usage Information

Application	WB, IP	Dilution	WB: 1:5000 - 1:10000; IP: 1:20
Reactivity	Human		
Source	Rabbit Monoclonal Antibody	MW	128 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: 5%.** 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 1:5000)**, 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/12435635/

Application Data

WB

Validated by Capsid Bio



Lane 1: Jurkat (AP-treated), Lane 2: Jurkat