Phospho-Ubiquitin (Ser65) Antibody [C22F3]

Catalog No.C15280 Batch:C1528003

Product Datasheet

Biological Description

Specificity	Phospho-Ubiquitin (Ser65) Antibody [C22F3] recognizes endogenous levels of Ubiquitin protein only when phosphorylated at Ser65.
Background	Ubiquitin is a highly conserved polypeptide that plays a central role in the ubiquitin-proteasome system, a key mechanism for regulating protein degradation within cells. Through a process known as ubiquitination, ubiquitin molecules are covalently attached to specific cellular proteins, marking them for degradation by the 26S proteasome. The ubiquitination cascade involves three main enzymatic steps: First, ubiquitin is activated by the E1 enzyme, forming a thiolester bond. This activated ubiquitin is then transferred to the E2 ubiquitin-conjugating enzyme. Finally, the E3 ubiquitin ligase facilitates the transfer of ubiquitin from E2 to the target protein by attaching it to the epsilon-amino group of a lysine residue on the substrate. The ubiquitin-proteasome pathway is critical for a wide array of physiological processes, including cell cycle regulation, differentiation, response to cellular stress, and apoptosis. Dysregulation of this pathway has been linked to various diseases. One regulatory mechanism involves the phosphorylation of ubiquitin at serine 65 by the kinase PINK1, which activates the E3 ligase Parkin—a key step in mitochondrial quality control. IκB, p53, cdc25A, and Bcl-2, are known substrates of the ubiquitin-proteasome system, highlighting its essential role in maintaining cellular homeostasis and controlling vital functions such as immune responses, DNA repair, and programmed cell death.

Usage Information

Application	WB, IP	Dilution	WB: 1:1000; IP: 1:100
Reactivity	Human		
Source	Rabbit Monoclonal Antibody	MW	
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 Lysis Buffer (containing Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail)，and homogenize the tissue at a low temperature.
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 Lysis Buffer (containing Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail) and put the sample on ice for 5 min.
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 Lysis Buffer (containing Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail) and put the sample on ice for 5 min.
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. 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. Reference Table for Selecting PVDF Membrane Pore Size Specifications
(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/10939967/ https://pubmed.ncbi.nlm.nih.gov/24751536/

Application Data

WB

Validated by Capsid Bio



Lane 1: PC-3, Lane 2: PC-3 (carbonyl cyanide 3-chlorophenylhydrazone, 30 μM, 6 h)