Phospho-PLCγ1 (Tyr783) Antibody [C6N17]

Catalog No.C15222 Batch:C1522203

Product Datasheet

Biological Description

Specificity	Phospho-PLCγ1 (Tyr783) Antibody [C6N17] detetcs endogenous levels of PLCγ1 protein only when phosphorylated at Tyr783.
Background	Phospho-PLCγ1 (Tyr783) represents the activated form of PLCγ1, a receptor-proximal signaling enzyme that hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate the second messengers IP_3 and DAG, thereby activating protein kinase C (PKC) and mobilizing intracellular Ca^{2+}. Structurally, PLCγ1 is composed of a catalytic core (X–Y domain) regulated by an autoinhibitory X–Y linker containing multiple domains: pleckstrin homology (PH), Src homology 2 (nSH2, cSH2), and SH3. In its basal state, the cSH2 domain interacts with the C2 domain of the catalytic core, physically blocking membrane access. PLCγ1 is broadly expressed in mammalian tissues, particularly in hematopoietic and epithelial cells, where it transduces signals downstream of receptor tyrosine kinases, immune receptors, and growth factor receptors. Phosphorylation at Tyr783, mediated by receptor-associated kinases, is the hallmark event that relieves autoinhibition: the phosphorylated Tyr783 acts as an intramolecular ligand that binds to the cSH2 domain, displacing it from the catalytic core and allowing exposure of a hydrophobic ridge required for membrane insertion and catalysis. This phosphorylation is essential because it locks PLCγ1 into its active conformation, stabilizes its interaction with the plasma membrane, and ensures robust propagation of receptor signaling, with dysregulated phospho-PLCγ1 implicated in oncogenesis and aberrant immune activation.

Usage Information

Application	WB, IP, FCM	Dilution	WB: 1:1000; IP: 1:50; FCM: 1:100 - 1:400
Reactivity	Human, Mouse		
Source	Rabbit Monoclonal Antibody	MW	155 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 1:1000), 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/35367415/ https://pubmed.ncbi.nlm.nih.gov/31889510/

Application Data

WB

Validated by Capsid Bio



Lane 1: NIH/3T3, Lane 2: NIH/3T3 (HPDGF-BB, 10 ng/ml, 15 min)