

Anti-Myosin Phosphatase/PPP1R12A Antibody

Catalog Number: PA1681

About PPP1R12A

PPP1R12A (Protein phosphatase 1 regulatory subunit 12A), also called MYPT1 (Myosin phosphatase target subunit 1), is an enzyme that in humans is encoded by the PPP1R12A gene. PPP1R12A is one of the subunits of myosin phosphatase. Sequencing analysis showed that human PPP1R12A contains 1,030 amino acids with a calculated molecular mass of approximately 115 kD. The PPP1R12A gene is mapped on 12q21.2-q21.3. PPP1R12A is the protein that regulates PP1 function in smooth muscle relaxation. The cellular MYPT1-PP1-delta -specific inhibitor CPI17 caused a loss of merlin function characterized by merlin phosphorylation, Ras activation, and transformation. Jin et al. concluded that PPP1R12A and its substrate merlin are part of a previously undescribed tumor suppressor cascade that can be hindered in 2 ways, by mutation of the NF2 gene and by upregulation of the oncoprotein CPI17.

Overview

Product Name	Anti-Myosin Phosphatase/PPP1R12A Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Myosin Phosphatase/PPP1R12A Antibody catalog # PA1681. Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg Thimerosal, 0.05mg NaN3.
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	O14974

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human PPP1R12A, identical to the related rat and mouse sequences.
Predicted Reactive Species	Chicken
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG





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Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 2ug/ml, Human Flow Cytometry, 1-3ug/1x10 ⁶ cells, Human



Anti-Myosin Phosphatase/PPP1R12A Antibody (PA1681) Images

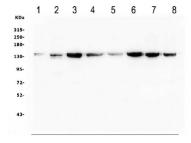


Figure 1. Western blot analysis of PPP1R12A using anti-PPP1R12A antibody (PA1681).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human Hela whole cell lysates,

Lane 2: human Jurka7 whole cell lysates,

Lane 3: human HEK293 whole cell lysates,

Lane 4: monkey COS-7 whole cell lysates,

Lane 5: human Raji whole cell lysates,

Lane 6: human K562 whole cell lysates,

Lane 7: human Caco-2 whole cell lysates,

Lane 8: human HepG2 whole cell lysates,

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-PPP1R12A antigen affinity purified polyclonal antibody (Catalog # PA1681) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for PPP1R12A at approximately 140KD. The expected band size for PPP1R12A is at 115KD.

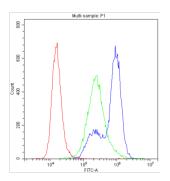


Figure 2. Flow Cytometry analysis of Hela cells using anti-PPP1R12A antibody (PA1681). Overlay histogram showing Hela cells stained with PA1681 (Blue line).The cells were blocked with 10% normal goat

serum. And then incubated with rabbit anti-PPP1R12A Antibody (PA1681,1ug/1x10⁶ cells) for 30 min at 20°C. DyLight® 488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

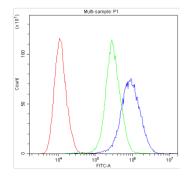


Figure 3. Flow Cytometry analysis of U251 cells using anti-PPP1R12A antibody (PA1681).

Overlay histogram showing U251 cells stained with PA1681 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-PPP1R12A Antibody (PA1681 ,1ug/1x10 6 cells) for 30 min at 20 $^\circ$ C. DyLight® 488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10 6 cells) was used as secondary antibody for 30 minutes at 20 $^\circ$ C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10 6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

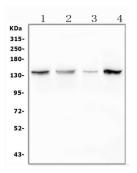


Figure 4. Western blot analysis of PPP1R12A using anti-PPP1R12A antibody (PA1681).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: rat brain tissue lysates,

Lane 2: rat C6 whole cell lysates,

Lane 3: mouse liver tissue lysates,

Lane 4: mouse NIH/3T3 whole cell lysates,

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-PPP1R12A antigen affinity purified polyclonal antibody (Catalog # PA1681) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for PPP1R12A at approximately 140KD. The expected band size for PPP1R12A is at 115KD.

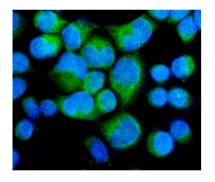


Figure 5. IF analysis of PPP1R12A using anti-PPP1R12A antibody (PA1681).

PPP1R12A was detected in immunocytochemical section of SiHa cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL rabbit anti-PPP1R12A Antibody (PA1681) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

1 Publications Citing This Product

1. PubMed ID: 26604905, The role of Rho/Rho-kinase pathway and the neuroprotective effects of fasudil in chronic cerebral ischemia

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